Received: 29 June 2016 | Revised: 5 August 2016 | Accepted: 11 August 2016

DOI 10.1002/ppap.201600108

FULL PAPER

PLASMA PROCESSES AND POLYMERS

# Effects of low temperature plasma on prostate cancer cells using the Bovie Medical J-Plasma® device

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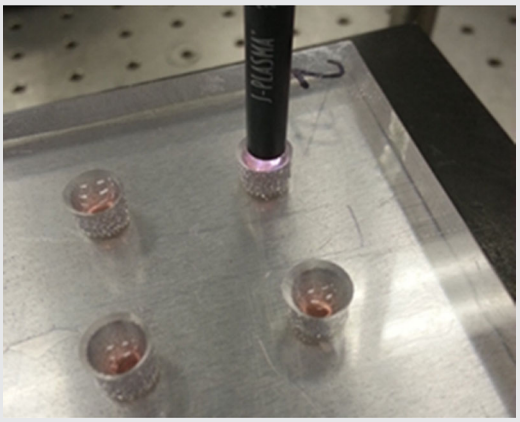
**Funding Information**
This work was supported by Bovie Medical Corporation.

The efficacy of low temperature plasma generated by an atmospheric pressure plasma jet, J-Plasma system (™Bovie Medical Corporation), is studied for its therapeutic effects against the DU145 prostate cancer cell line. The DU145 cells in complete culture media were treated with different exposure times and different J-Plasma system parameters. The optimum settings of the J-Plasma system were identified based on the cancer cell viability at 0, 12, 24, 48, and 72 h post-low temperature plasma treatments. The results indicate that J-Plasma killing and cell viability was exposure time dependent and induced a reduction in cell proliferation and delayed killing effect.




**KEYWORDS**
cancer, DU145 cells, J-Plasma, low temperature plasma, prostate

## 1 | INTRODUCTION

In the last decade, non-equilibrium, low temperature, atmospheric pressure plasmas (LTP) have been shown to be an effective medium for destroying different types of cancer cells.[1–26] LTP produces charged particles, electric fields, and reactive species that can act on biological cells individually and in synergy with each other. This interaction of plasma factors leads to various biological outcomes ranging from lysing bacterial cells, to enhancing the proliferation of fibroblasts, to inducing apoptosis or necrosis in cancer cells. Recently, the killing of cancer cells became one of the most active research topics in the field of plasma medicine. Investigators showed that LTP preferentially affects cancer cells by interrupting the cell replication cycle,[13] and/or by activating caspases (leading to apoptosis),[6] and/or by elevating intracellular reactive oxygen species (ROS) which can lead to DNA damage.[2] Extensive in vitro work showed that LTP can destroy various cancer cell lines such as leukemia, carcinoma, glioma, and melanoma, for example.[1] In vivo work showed that LTP can reduce tumor size and its effect extends below the surface layer.[2,7,10,18]

All these promising results led to intensive research to understand the detailed mechanisms that enter into play when biological cells are exposed to LTP, and to seek ways whereby LTP can be more efficient in eradicating cancerous cells. In this manuscript, we report results on the viability of prostate cancer cell line DU145 after exposure to LTP jet generated by Bovie Medical Corporation's J-Plasma system, a device termed J-Plasma. J-Plasma is energized by pulsed RF power and produces a relatively low temperature helium plasma jet. Unlike the more common local discharge mode cold plasma applicators, J-Plasma utilizes a direct discharge mode with a continuous discharge conduction path between

the applicator and the target surface, and operates by displacement currents.

The DU145 cancer cells suspended in culture media are exposed to the plume generated by J-Plasma for various treatment times and the viability of the cells is assessed immediately after treatment and at 12, 24, 48, and 72 h post-plasma treatment. The cell viability evaluation shows that DU145 cells in culture media are susceptible and killed using 0.5–4 min of exposure to J-Plasma.

## 2 | EXPERIMENTAL SECTION

### 2.1 | Bovie's J-Plasma®

J-Plasma is a form of cold plasma that relies on a direct discharge mode of operation. Unlike the more common local discharge mode of cold plasma applicator, where a ground ring around the applicator nozzle tip establishes a plasma discharge primarily within and local to the applicator, a direct discharge mode forms a continuous discharge path from the applicator directly to the target site. This direct discharge plasma beam is produced by flowing helium over a sharp conductive point that is held at high voltage and high frequency. The J-Plasma generator operates at a maximum of 6.5 kV peak to peak, producing a maximum of 40 W, at a frequency of 492 kHz. The helium flow rate can be adjusted up to a maximum of 5 L $min^{-1}$. Typical currents carried by the plasma beam range from a few milliamperes to a few tens of mA, depending on the power setting. In addition, the power level can be fine-tuned by duty cycling the on/off ratio of the power. Figure 1 is a photograph of the J-Plasma system.

A wide range of physiological effects can be selected by various combinations of electrical power, which provides a heating effect, and the helium flow rate, which provides a cooling effect. For example, if a high power setting is combined with a low helium flow rate, a hot intense ablative beam is formed useful in removing tumors, for example. By contrast, if a relatively low power setting is used with a high helium flow rate, a gentle, tissue tolerable beam results, which can be used for wound healing, disinfection, and cancer treatment studies.

Due to the continuous direct discharge path between the applicator and the target site, interaction between the plasma beam and the surrounding atmosphere is enhanced. This results in improved production of radical oxygen species, radical nitrogen species, and radical oxy–nitrogen species, which play an important role plasma cancer treatment.

### 2.2 | Cancer cell line maintenance

The cancer cell line being investigated is a prostate cancer cell line that had metastasized and was originally isolated from a patient's brain. The cell line was obtained from the American Type Tissue Culture (ATCC-DU145) and was conditioned to grow in Roswell Park Memorial Institute (RPMI)-1640 media supplemented with 10% fetal bovine serum, 1% antibiotic mixture of penicillin/streptomycin, and 1% glutamine. The cells have been previously investigated using the Plasma Pencil[1] and function as model cancer cell line to test the efficacy of the Bovie Medical J-Plasma System. The cancer cells were maintained, passaged, and incubated post-treatment at 37 °C and 5% $CO_2$.


**FIGURE 1** Image of the Bovie Medical J-Plasma® System showing power source, handheld plasma applicator and different tips

### 2.3 | Evaluation of the pH change in water and media after treatment with J-Plasma®

Due to the use of displacement currents in the direct discharge mode of J-Plasma, a conductive aluminum tray was grounded and used to treat liquid media. The aluminum trays were fabricated with well diameters equivalent in size to the 24-well culture plates ($12 \times 6\ mm^2$ depth). The initial set of experiments to determine pH change was conducted using water or RPMI-1640 as liquid substrates in an aluminum tray. Approximately 1 ml of liquid was filled to the top of the well and treated with J-Plasma (Figure 2). The duty cycle was set at about 17% with T-on:10 ms and T-off:50 ms; the applied power was set at either 16 or 8 W. The gas flow rate was constant at 4 slm and the nozzle of the J-Plasma handheld device was kept at 1 mm above the surface of the liquid. The temperature of the water and RPMI-1640 was 23 °C and the starting pH of both water and RPMI-1640 was 7.4. The results of the measurements are described in Table 1 of the Results section.

### 2.4 | Evaluation of the J-Plasma® thermal activity on a copper surface

The temperature of the plasma jet generated by the J-Plasma system was characterized by using a thin copper sheet that

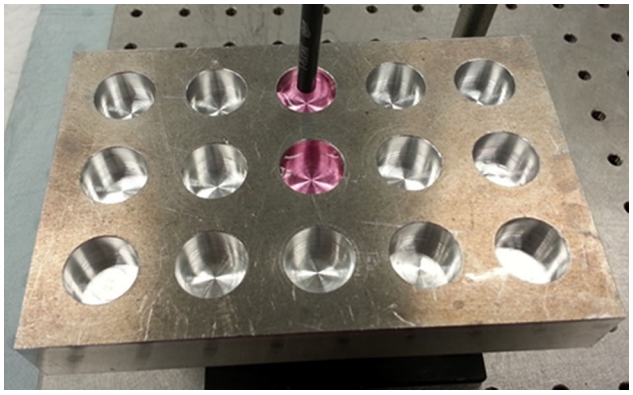


**FIGURE 2** Image of the aluminum tray that was used to treat either liquid (water or RPMI-1640) or DU145 cells in complete RPMI media. The size and dimensions of the wells were equivalent to standard 24-well cell culture plates

was grounded with a thermocouple attached to the bottom surface of the copper sheet to measure temperature fluctuations digitally. The thin copper sheet was placed on an adjustable stand while the nozzle of the J-Plasma was set at ~2 mm distance above the thin copper sheet. Table 2 contains the change in temperature with specific J-Plasma parameters.

## 2.5 | DU145 prostate cancer cells in complete RPMI media were exposed to J-Plasma®

The DU145 cells were grown in the complete RPMI-1640 growth media in a 75 $cm^2$ vented cell culture flask until high confluence of cells were obtained. The DU145 cells were trypsinized by Trypsin solution (1×) to detach the cells from the flask. The suspension contained ~$10^6$ cells $ml^{-1}$. This solution was diluted 1:10 to obtain $10^5$ cells $ml^{-1}$ and each sample with a volume of 1 ml containing $10^5$ cells was treated by J-Plasma with different exposure times directly in the wells of the grounded aluminum tray. Subsequently, the cells were transferred to a 24-well plate, incubated at 37 °C and 5% $CO_2$, and cell viability was determined through direct counting of live versus dead cells via trypan blue exclusion assay immediately after J-Plasma treatment and 24 or 48 h post-treatment (Figure 3). The trypan blue exclusion assay reveals live cells that are transparent because the dye cannot penetrate into the intact cell membrane while dead cells are stained blue as the dye crosses the membrane barrier through pores found in dead cells. The plasma exposure times were 1, 2, and 4 min, and the untreated cells (indicated by 0 min treatment) were used as the control for the cell viability assays. The 0 min treatment samples were treated by helium gas flow alone without ignition of plasma for up to the max exposure time (4 min) to show that helium gas does not have any effect on cell viability.

**TABLE 1** The pH measurements of water or RPMI after J-Plasma treatment

| Duration (s) | Water pH 16 W | Water pH 8 W | RPMI pH 16 W (control 7.6) |
|---|---|---|---|
| 30 | 7.3 | 7.3 | 7.6 |
| 60 | 7.0 | 7.3 | 7.66 |
| 120 | 6.9 | 7.0 | 7.6 |
| 240 | 6.7 | 6.8 | 7.4 |

**TABLE 2** The temperature at specific power and duration of J-Plasma treatment

| Duration (s) | Power: 16 W (°C) | Power: 8 W (°C) |
|---|---|---|
| 30 | 27 | 35 |
| 60 | 29 | 36 |
| 120 | 42 | 38 |
| 240 | 45 | 38 |

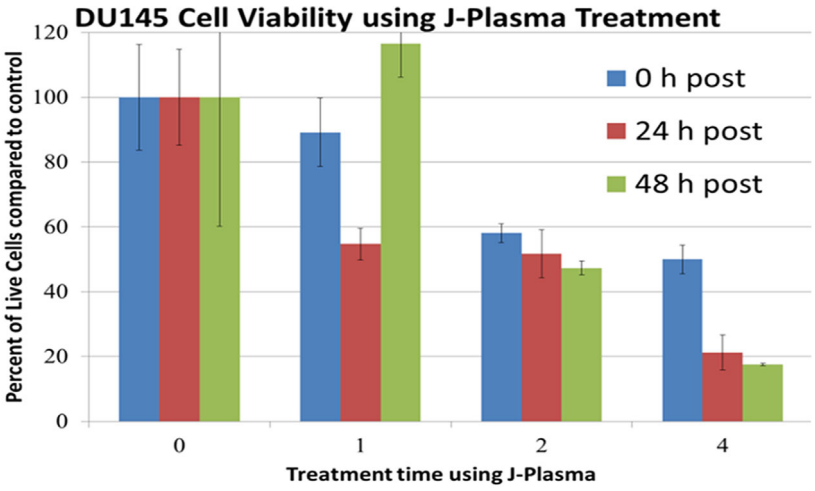
## 2.6 | MTS assay indicates inhibition of proliferation of DU145 cells after J-Plasma® treatment

The general trends seen using the trypan blue exclusion assay are that increasing the time of J-Plasma treatment resulted in increased killing of cancer cells. The direct counting method was useful to determine killing of cancer cells and indicated that a single treatment results in long-term killing. As an alternative, the MTS assay was conducted to determine the cell viability or inhibition of cell proliferation (Figure 4). The CellTiter 96® AQueous One Solution Cell Proliferation Assay was used in the MTS assay. The same DU145 cell suspension was used but at one-tenth the original volume in wells of 96-well plates. The procedure of treatment was identical as the trypan blue exclussion assay and experiments were performed to determine the change in concentration of cells using a colorimetric method that was measured in a microplate reader (AgileReader, ACTGene Inc.) at 490 nm wavelength.

# 3 | RESULTS AND DISCUSSION

## 3.1 | Evaluation of the pH change in water and media after treatment with J-Plasma®

In the initial stages of this investigation, the J-Plasma system was used to treat water and RPMI-1640 media in order to determine any changes in pH. Some studies involving plasma activated water and media have described distinct changes in the pH of the treated liquid. In order to investigate this phenomenon, 1 ml of water or RPMI-1640 was treated with J-Plasma in a grounded aluminum tray (Figure 2). The duty cycle was set at about 17% with




| Hours (post-treatment) | 0 min | 1 min | 2 min | 4 min |
|---|---|---|---|---|
| 0 | 100 % | 89 % | 58 % | 50 % |
| 24 | 100 % | 55 % | 52 % | 21 % |
| 48 | 100 % | 117 %* | 47 % | 18 % |

**FIGURE 3** Percent viability calculated by the Trypan Blue exclusion assay to determine live versus dead cells immediately after J-Plasma treatment and 24 or 48 h post-exposure

The pH analysis indicates that the water was prone to greater pH effects compared to the RPMI-1640 media. When the applied power was set at 16 W, the pH change of the water was most dramatic at 0.6 with the 4 min (240 s) exposure time. Even when the power setting was at 8 W, the pH change was 0.5 at the highest exposure time. This indicates that independent of the power setting, the water pH fluctuates dramatically. However, using the highest power setting of 16 W and treating the RPMI-1640 media with the longest exposure time (240 s), there was very little change in the pH. The RPMI media pH was not dramatically affected by the plasma indicating that the pH effect is negligible in the culture media. This is a characteristic common among media that is buffered. Therefore, treatment of cells in water may have a pH side-effect. While using RPMI, the changes are negligible due to its innate ability to buffer the media. Therefore, the J-Plasma effects are due to the plasma factors and not the change in the pH, since it is negligible.

T-on:10 ms and T-off:50 ms; the applied power was set at either 16 or 8 W, and the helium flow was 4 slm. The temperature of the water and RPMI-1640 was determined to be approximately at room temperature (23 °C), and the starting pH of the water and RPMI-1640 was 7.4.

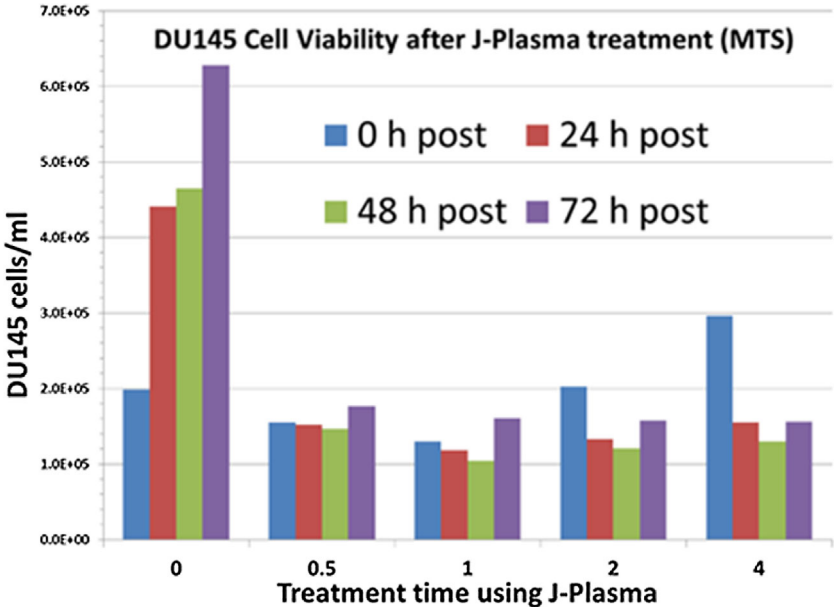



**FIGURE 4** MTS assay showing inhibition of proliferation and killing of DU145 cells. DU145 cell counts were determined immediately after J-Plasma treatment or at 24, 48, or 72 h post-exposure

## 3.2 | Evaluation of the J-Plasma® thermal activity on a copper surface

The temperature of the plasma jet was determined by using a thin copper sheet that was grounded with a thermocouple attached to the bottom of the thin copper sheet. This process allowed for the temperature to be accurately measured using a digital thermocouple. In order to achieve a biologically tolerable temperature regime, the power was set at 16 or 8 W with a similar duty cycle as above and the results are shown in Table 2.

The results of the temperature analysis using the power settings of 16 W and duty cycle set at 50% (T-on:10 ms and T-off: 10 ms) resulted in a dramatic increase in temperature from 23 °C to 72 °C (data not shown), which is not a biologically tolerant temperature. The results of the temperature experiment revealed that the ideal power setting to obtain a tolerable treatment regime is an applied power of 8 W or below. Therefore, these findings were essential for us to establish a base line to use with the mammalian cells. In order to alleviate any temperature effects, the J-Plasma power was set at 8 W when the DU145 cancer cells were treated with the J-Plasma.

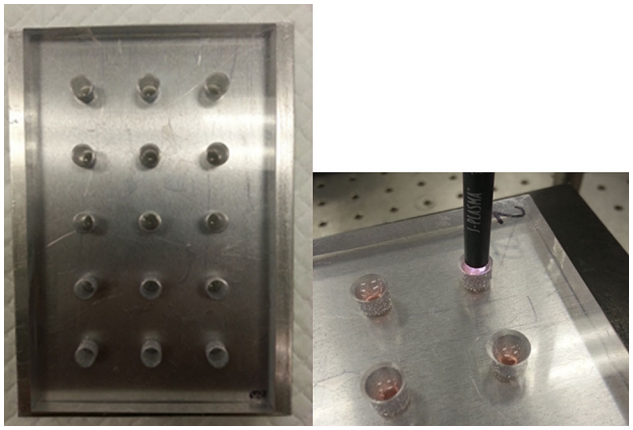


**FIGURE 5** The fabricated wells with an aluminum base and plexiglass wells. The volume was decreased to 300 ml and the plasma was directly treating the entire surface of the media with cells

## 3.3 | Cell viability

In order to ascertain the effect of the J-Plasma on DU145 killing and proliferation, the prior investigations on temperature indicated the ideal plasma parameters settings which were subsequently used in this next section: applied power of 8 W, duty cycle set at around 17% with T-on:10 ms and T-off: 50 ms, helium gas flow at 4 slm. The cell viability was assayed at 0, 12, 24, or 48 h post J-Plasma treatment using trypan blue exclusion assay and the MTS assay (see Figures 3 and 4, respectively). The direct counting method was useful to determine killing of cancer cells and indicated that a single treatment results in long-term killing. The normal growth of DU145 is indicated by the immediate cell counts at 0 min treatment. Those values were set as the norm when comparing the amount of cells at 24 or 48 h post-plasma treatment. The general trend is that increased exposure with a maximum effect at 4 min exposure kills the most cells immediately after plasma exposure (0 min post) and 24 h as well as 48 h. This is indicative of the reactive species that have both immediate effects based on the short lived species and the long-term effect based on the species that continue to act upon intracellular signals in the cancer cells. There was a slight increase of samples treated with 1 min of J-Plasma indicating the rebound of cells at the lower dose in this experiment.

Automated MTS counts using a microplate reader, revealed lower cell counts at exposure times of 0.5–4 min when compared to the control 0 min treatment (see Figure 4). These results indicate decrease of cells (fivefold) and inhibition of proliferation when compared to the control cells that show a continued activity to proliferate (0 min columns) at 24, 48, and 72 h post-treatment.

In order to observe significant difference in cell proliferation and cancer cell killing, a fabricated plate containing wells that can hold volumes of 300 ml were made. The main purpose of this assay was to increase the area of treatment by the plasma generated with the J-Plasma. The fabricated wells were made of Plexiglass with an aluminum sheet attached with epoxy on bottom. The holes in the plexiglass were made with a diameter of 6 mm and a depth of 12 mm. The total volume that could be placed in each well was ~350 ml (see Figure 5). As the wells were much smaller the volume of liquid was smaller. However, the cell concentration was kept the same at $\sim 2 \times 10^5$ cells $ml^{-1}$. The plasma parameters of Power: 8 W, T-on:10 ms and T-off:50 ms, and He: 4 slm were used in this assay and the cell viability revealed excellent effect at even lower exposure times (see Figure 6). Although there was minimal change immediately after or 12 h post-plasma exposure, we observed significant inhibition of cell proliferation at 24 h post-plasma treatment. These results indicate that smaller treatment areas would be ideal targets to kill the most cancerous cells. In addition, the time of 30 s was significant using the directed plasma approach.

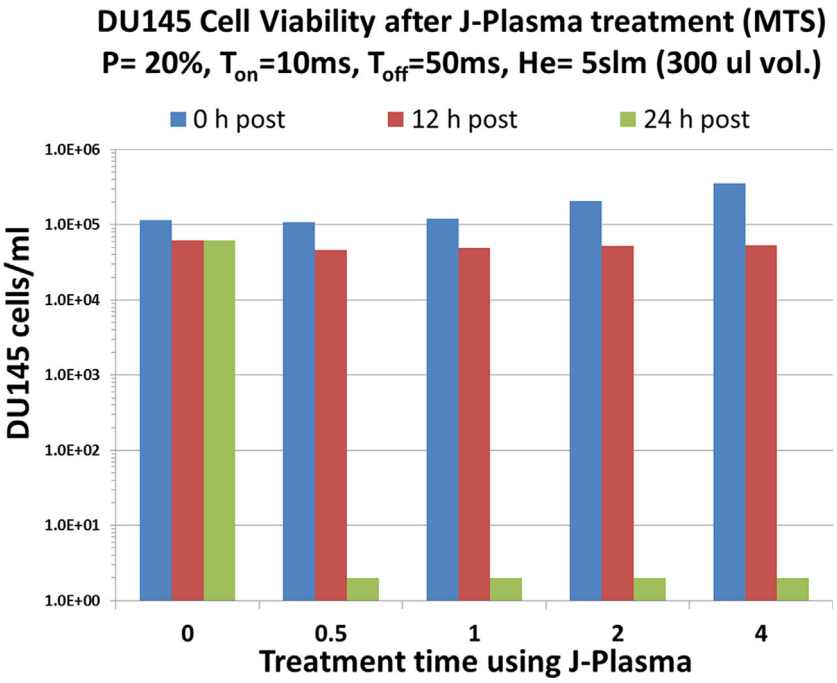



**FIGURE 6** The MTS assay of DU145 cells treated with 0.5–4 min of J-Plasma in smaller fabricated wells

## 4 | CONCLUSION

The results indicate that the J-Plasma® has the ability to kill cancerous cells as shown by the trypan blue assay. Furthermore, the J-Plasma device is capable of inhibiting cell proliferation as indicated by the MTS assay.

The ideal biologically tolerant J-Plasma parameters are Power: 8 W, T-on:10 ms and T-off:50 ms, and He: 4 slm, and reveal excellent killing at 30 s of duration against DU145 cancer cells in liquid media. As a potential therapeutic, the 30 s window would be practical in cancer treatment and provides a usable time frame to treat areas either undergone with surgical removal of cancerous lesions or as a natural treatment to inhibit the progression of tumors. The work presented here shows the potential of the J-Plasma as a method to kill and inhibit the proliferation of prostate cancer cells.

## ACKNOWLEDGMENTS

The authors would like to thank S. Mohades for technical assistance. This work was partly supported by the Bovie Medical Corporation.

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