Cold Atmospheric Plasma generated by FE-DBD Scheme cytotoxicity against Breast Cancer cells

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Abstract
Cold atmospheric plasma (CAP) occupied a wide area in cancer therapy field, this type of plasma is very close to room temperature. This study would illustrate the effect of CAP on the breast cancer cells in vitro. AMJ13 Iraqi human breast cancer cell line was used for the in vitro study. Floating Electrode-Dielectric Barrier Discharge (FE-DBD) system is used for this purpose, the cold plasma output power ranged 6 - 57 W and temperature of the produced plasma ranged 32 - 45^{0}C.

A cold plasma produced by the device was tested against the breast cancer cells. The induced cytotoxicity is found to be 58.07% in AMJ13 with severe morphological changes including nuclear condensation which refers to apoptotic death pathway. The current method is promising for breast cancer therapy.

Keywords: Cold atmospheric plasma, FE-DBD, breast cancer therapy, cytotoxicity, AMJ13.

Introduction

Plasma is the fourth state of the matter represented by quasi-neutral gas consisting of neutral and charged particles that demonstrates the collective behavior. It forms approximately 99% of universe matter as electrified gases of dissociated positive ions and negative electrons of the atoms, so it displays a fact of atmospheres, interstellar hydrogen, gaseous nebulae and stellar interiors are plasmas.[21] Plasma is a gassy mixture of electrons and positive or negative ions, it can be partially ionized as the plasma generated in fluorescent lamps, or fully ionized such as the plasma generated in the sun.[18]

There are two kinds of plasma, thermal and non-thermal which are based on the temperature of the electrons with respect to the other particles (ions and neutral particles). In thermal plasma, the electrons and heavy particles are in thermal equilibrium (have the nearly the same temperature), while in nonthermal plasma, the heavy particles are nearly at room temperature while the electrons get a much higher temperature. Conventional[6] thermal plasma temperatures greater than 3000^{0}C at the target, make it typical for mineralogy but unsuitable[7] for living tissue treatment. Nonthermal plasma is also called cold plasma and at the point of application, cold plasma has a temperature of less than 40^{0}C, making it suitable for living tissue treatment.[8,15]

Cold plasma can be generated in low and high pressure (atmospheric pressure). The possibility to generate cold plasma in atmospheric pressure (which is called Cold Atmospheric Pressure CAP) acquire the plasma additional feature such as vacuum champers and expensive vacuum systems.[14] The most important cold plasma in medicine was cancer therapy, which would be focused on this study. Non-thermal plasma has been tested for its ability to treat various types of cancers effectively by selectively killing cancer cells while leaving normal tissue unharmed.[5,9] The aim of the current research is to study the effect of CAP as breast cancer therapy *in vitro* models using locally constructed FE-DBD.

Material and Methods

Floating Electrode Dielectric Barrier Discharge FE-DBD: This devise was designed and manufactured by our team. The constructed FE-DBD system consists of two essential parts; probes and high voltage source. The probe consists of two basic parts, (i) The base of probe: (ii) The external probe. The High voltage source was designed especially for the proposed system. The circuit was based on a special kind of transformer called Flyback Transformer (FBT). This type of transformer is able to raise the voltage to several thousand volts. The circuits are fed by a continuous voltage equal to 12V.

Cytotoxicity Assay: This study used Iraqi human breast cancer AMJ13 cell line. For tissue culture, a microplate of 96 (12×8) well was used, each well was used to seed 10,000 cancer cells, to be incubated at 37^{o}C for 24 hours until monolayer was achieved as observed by inverted microscope. Cells were exposed to CAP using proposed plasma generator system with small diameter probe for three different intervals: 5 sec, 10 sec and 15 sec and control wells were left without treatment. The described steps were done in triplicate and re-incubated at 37^{o}C for different intervals, 24hrs, 48hrs and 72hrs.

After incubation, the growth medium was decanted off. These steps were repeated three times to confirm the veracity. MTT (100µL of MTT diluted by 1mL of free serum medium) was added (100µL of diluted MTT for each well) before it was incubated for 2 hours then decanted off and DMSO was added (50µL for each well) and re-incubated for 40 min at 37^{o}C. During re-incubation duration, the assay is pulled out from cells mitochondrion which gives an indication for the percentage of inhibited cells according to the violet darkness of resulted solution, therefore, the resulting solution of control group has the darkest violet

color.[4,11] Microplate reader was used to read the results at 580 wavelength to calculate the percentage of the live cancer cells (inhibition rate), then the mean value is computed for each group.[1,22] The calculated mean of inhibition percentages of 72 hours was recorded separately for results analysis.

Clonogenicity assay and morphological analysis: The AMJ13 breast cancer cells were seeded in 6 wells tissue culture plates at a density of $5x10^5$ cells/mL and incubated overnight. After confluency, the cells were exposed to CAP in three different time intervals 5, 10 and 15 seconds. The wells were fixed and stained using crystal violet and observed under inverted microscope and photographed by digital camera.[23]

Statistical Analysis: The experiments results were analyzed by GraphPad Prism v7.0 to estimate the behavior of the proposed CAP system impact on breast cancer cells *in vitro*.[10]

Results and Discussion

Cytotoxicity Assay: The *in vitro* results of cytotoxicity against AMJ13 breast cancer cell line were evaluated under different doses of CAP, 5sec, 10sec and 15 sec, to measure the impact of plasma on the cancer cell line. The maximum cytotoxicity was achieved at 10 seconds of the exposure after 48hrs of incubation. The percentage of cytotoxicity was 58.07% (figure 2).

Morphological study: CAP treated cells showed less cell number due to detachment in all fields observed. Moreover, there was condensed nuclei which refer to apoptotic cell death and this picture shown in the three times of exposure (figure 3). Control untreated breast cancer cells continue to grow to confluency in the observed fields under inverted microscope and photographed using digital camera.

Clonogenicity Assay: We conducted clonogenic assay to evaluate the growth inhibition activity of the CAP therapy against AMJ13 cell line. The ability of cancer cell to form colony is investigated to measure the activity of CAP. CAP induced reduction on the colony formation of the human breast AMJ13 cells at 10 seconds of exposure (fig. 4). Control untreated cells showed complete cells growth to confluency. These results showed that CAP treatment can reduce breast cancer cells proliferation significantly

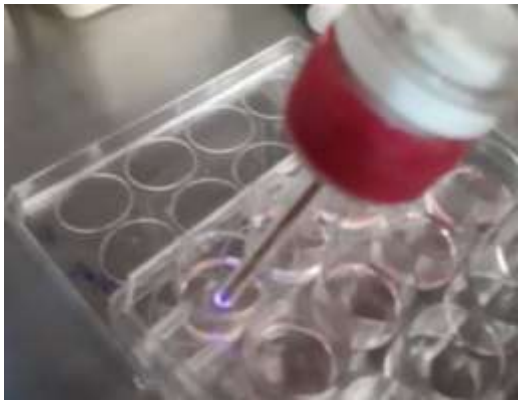

Fig. 1: CAP exposure tissue culture

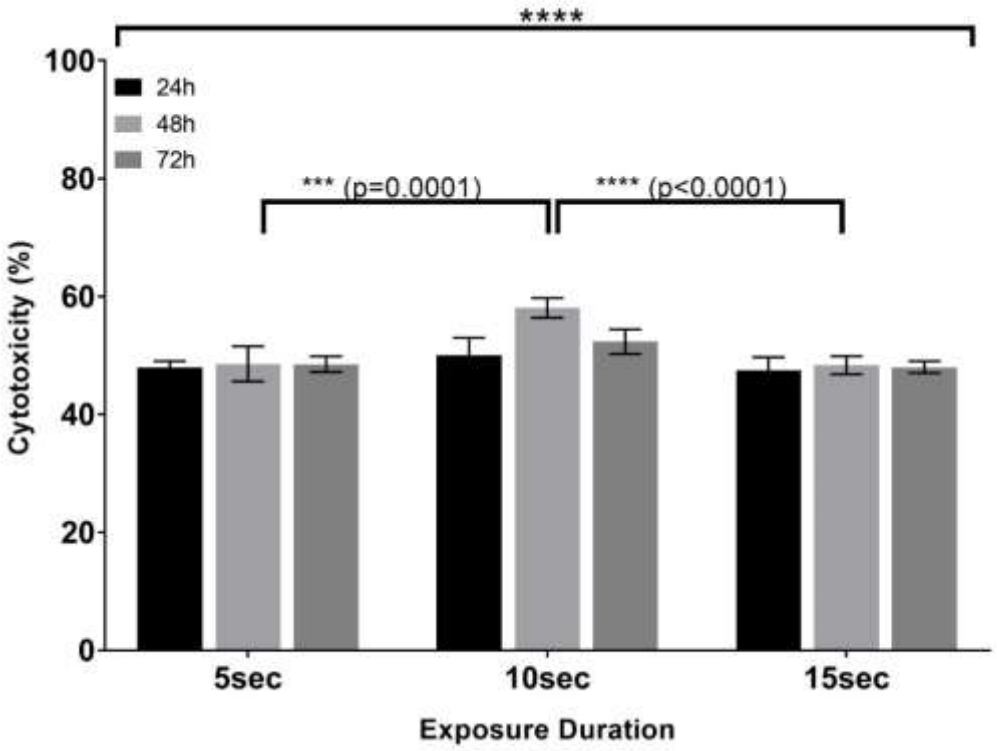

Fig. 2: Cytotoxicity of CAP against Breast cancer AMJ13 cell line in vitro using three different exposure times 5, 10 and 15. 10 seconds of exposure shown to induce the best effect at 58% cytotoxicity.

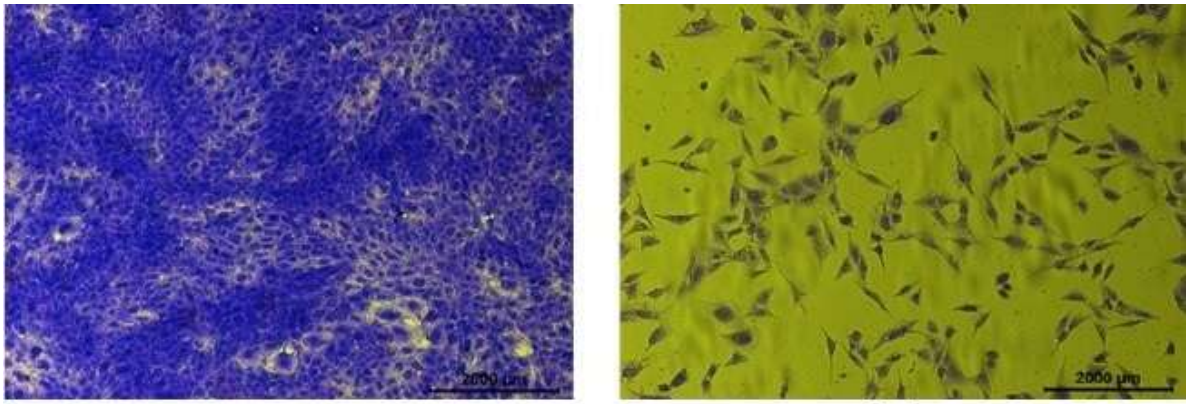

Fig. 3: AMJ13 breast cancer cell line stained with crystal violet stain and observed under inverted microscope. (A) control untreated cells showed confluency. (B) CAP-treated cells for 10 seconds showing cell detachment and less cell number as well as nuclear condensation.

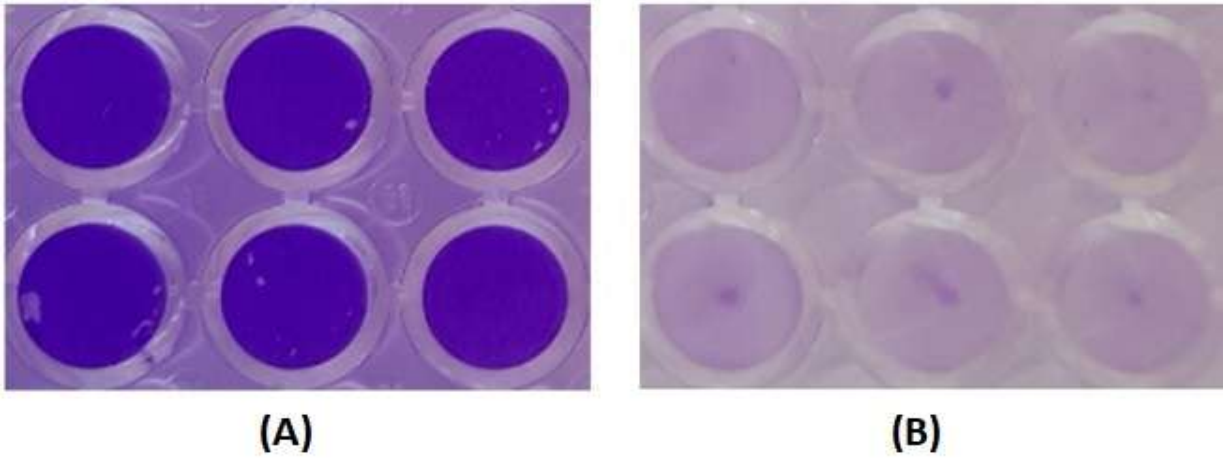

Fig. 4: The ability of cancer cell to form colony is investigated by clonogenic assay to measure the activity of CAP. (A) CAP induced reduction on the colony formation of the human breast AMJ13 cells at 10 seconds of exposure. (B) Control untreated cells showed complete cells growth to confluency.

Cancer currently is of the significant problems in Iraq.[3] Breast cancer is the utmost threatening cancer in women.[2] There is pressing need to find cure for this malignant disease.[4] In our study, we used cold atmospheric plasma produced by lab designed FE-DBD; the power of the output plasma was 6.6 W, this value of plasma power was very convenient to inhibition of cancer cell without causing any damage to normal cell.[17,24] The results of the study showed the effect of selective cold plasma on the inhibition of breast cancer cells without affecting normal cells.[13] Our investigation proves the strong impact of cold plasma therapy on cancerous cells *in vitro*. Previous research has shown different possible mechanisms for cold plasma's impact by using plasma jet.[12,20]

Several apoptotic and oxidative stress pathways genes were deregulated in tumors treated with cold plasma. Moreover, the selective influence of cold plasma on several cell types suggests that it is possible to find the right conditions with plasma therapy hitting only cancer cells, leaving normal cells essentially unharmed. Interestingly, these results were translated to *in vivo* models of cancer therapy, with marked reductions in tumor volumes and improved survival treatment by cold plasma without thermal damage. Thus, the development of cold plasma treatment will cause a paradigm shift in cancer therapy. These astonishing preliminary results suggest that the cold plasma can selectively treat some cancer cells such as melanoma, colon and bladder.[12,16,19]

In brief, the current proof-of-concept research shows new *in vitro* response of cancer cells upon treatment with cold plasma FE-DBD. The best-known cold plasma outcome is plasma-induced apoptosis which can have significant associations in cancer therapy by restricting the affected area of the tissue.

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