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Feasibility of pulse power application in cell biology and cancer treatment: a review

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In this review paper after various reported data from 2000 to 2016 published in IEEE and science direct, are analyzed and discussed to explore the feasibility of pulsed power application in cell biology. Targeting inhabitation of cancer/tumor proliferation treatment as main fields, some other associated topics e.g. effect on plasma membrane, apoptosis induction etc. are investigated in vitro and in vivo, it summarizes, HOW the nano second Pulse Electric Field (nsPEF) affects eukaryotic cells that are healthy as well as that are affected by tumor/cancer i.e. unhealthy. Term electroporation came into the picture which stands for opening pore in cell membrane using pulse of electricity, to introduce DNA or chromosomes into bacteria or other cells. Width of the applied electrical pulse used also caused different cellular effects, pulses longer than 100μs, in electroporation, delivers DNA, protein, small drugs and fluorescent indicators across the plasma membrane and causes moderate levels of Phosphatidyl Serine (PS) translocation at the Plasma Membrane, while shorter pulses less than 1μs are central to intracellular effects such as apoptosis induction (programmed cell death) and higher levels of Phosphatidyl Serine (PS) translocation. In addition, nsPEFs acts as cellular stress that introduces translational suppression. Ultra-short electricity i.e. nsPEFs can reach intra cellular component directly without membrane destruction causes apoptosis induction (programmed cell death). Also it has been found that direct current produced by applied voltage induces specific biological healing of tissues near the electrodes, also the effect of current is same as in ionizing radiation of tumor therapy. Chemotherapeutic drugs along with nsPEF reduces dose of both types of treatments. Also, nsPEFs causes transient activation of signaling pathways involving Mitogen-Activated Protein Kinases (MAPKs). Now days, nsPEFs are recognized as unique tool in life science.

Keywords: *Nano second pulsed electric field, apoptosis induction, electroporation.*

1.0 INTRODUCTION

CANCER is most dangerous disease, since few decades, although there are many types of treatments by which cancer can be eliminated successfully e.g. surgery, radiation therapy, chemotherapy, targeted therapy etc., but of those either there is side effect or risk of life by infection or heating effect. Here it is an investigation of novel method of cancer treatment i.e. pulse power treatment in which damaged or unwanted eukaryotic cells (cells found in plant and animal) are removed using high intensity electric field of very short duration. Electroporation a phenomenon (making pores in plasma membrane), in eukaryotic cells exposed to high voltage electric field pulses having duration of the several ms to few μs time scale, is investigated using a numerical model for deep analysis [1]. The electroporation induced by electric field can be used for delivering DNA, small drugs, proteins, and fluorescent indicators across the plasma membrane and for other medical applications. There are two

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type of electroporation named conventional electroporation and supraelectroporation. Conventional electroporation occur when pulse is longer than 100µs period and field intensity is in between 0.1 to 1 kV/cm. This causes moderate levels of phosphatidylserine translocation at the plasma membrane and also conventional electroporation is reversible means after removal of field cell reverts in its original states. Supra-electroporation by 10 kV/cm to 300 kV/cm pulses and shorter than 1µs, affect at inner members of cell and cause intracellular effects such as apoptosis induction which is irreversible and causes permanent change in cell structure and also higher levels of phosphatidylserine translocation [2]. Conventional electroporation mainly affects at the plasma membrane, but endoplasmic reticulum is also affected somewhat but not dangerously. Biofouling, naturally growth of some aquatic species on any wet surfaces. Prevention of the growth of aquatic species can be achieved by using pulsed electric field of several kV/cm amplitude and some nano second duration on surfaces [3]. NsPEFs are also can be used for bacteria and decontaminating liquids potentially [4]. It has been demonstrated that if the duration of electric pulses are very short and the electric field sufficiently high, the time required for charging of the plasma membrane apparently is not reached, but subcellular vesicular membranes are breached [5]. This increases the possibility that nsPEF can be used to develop induced apoptosis (programmed cell death) in mammalian cells by affecting cell structures internally. This phenomenon can be used to kill the cancer cells in mammalian or in vivo, it is confirmed in vitro [6].

2.0 MATERIAL AND METHOD

2.1 Pulsed Power Generator

In few papers authors have used nanosecond pulsed power generator as shown in figure 1 [7], [8], & [9]. The generator has a capacitor bank C1, tesla transformer (produces high-voltage, low-current, and high frequency electricity), pulse forming line, gas gap switch with a short gap, and transmission line for pulse. A personal computer is used to control the DC power supply and a triggered spark gap switch system. A nanosecond pulse width was accomplished using a combination of a short pulse forming line and a highly pressurized gas spark gap switch with a very short gap separation. The natural impedance of the transmission line was about 86 Ω. Although any suitable high voltage pulse generator can be used for experiment.

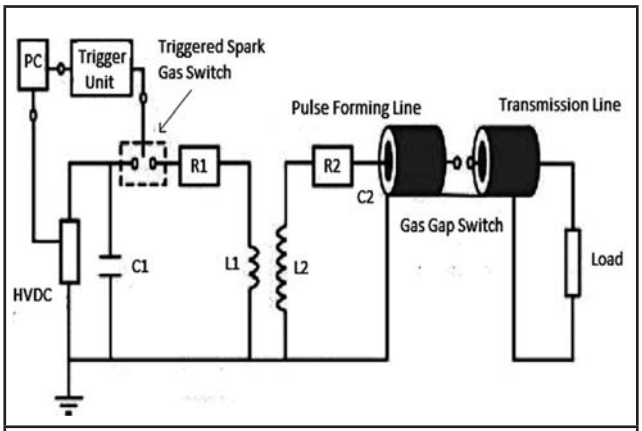

FIG. 1. PULSE POWER GENERATOR (SCHEMATIC DIAGRAM)

2.2 Embryonic Chick Assay

In this test, tumor cells are implanted with capillary on chorioallantoic membrane in chicken egg. Using with mouse breast adenocarcinoma cells, solid tumor is formed, EMT6/KU. There are several advantages embryonic chick assay as-

- Can be performing many tests with low cost.
- Low infection defense ability.
- Can be applied to various viruses and rickettsia.

The effects of nsPEFs, on solid tumors were investigated using embryonic chick assay [7], [8], & [9].

2.3 Cell Culture

In *va*rious reports various cell cultures were used for application of nsPEFs in vitro and investigated.1-Human Jurkat cells were cultured according to description given by ATCC (American Type Culture Collection). The fibrosarcoma B10.2 was

cultured in RPMI media (Roswell Park Memorial Institute media) with 5% FBS (Fetal bovine serum) [5]. SKOV3 cancer cells were cultured in RPMI-1640 substrate (GIBCO Company), which contained 10% calf serum, 100U/ml penicillin and 100U/ml streptomycin. Cultured in a 37 ^{0}C, 5%CO2 humidified incubator, these cells bred one time every 4-5days [10]. 2-Mouse Hepa 1-6, rat N1S1 and human HepG2 cell lines were obtained from ATTC, cultured as recommended and analyzed in log phase growth. Cells were exposed to one, three or ten pulses with 60 ns or 300 ns durations at various electric fields in cuvettes with a 0.1 cm gap at 7.7 $\times 10^6$ cells/ml [11]. 3-HeLa S3 (ATCC CCL-2.2) cells, a substrain of HeLa, were used in this study. Although originally an adhesive cell, HeLa S3 can be cultivated in a state of suspension. They were maintained in an alpha modified Eagle's minimum essential medium (αMEM, GIBCO) containing 10% fetal bovine serum (FBS, GIBCO) and antibiotics at 37 ºC in a humidified, 5% CO2 incubator. The cultured cells adhering to the bottom surface of the plastic dish (100 mm; FALCON) were washed twice using phosphate buffered saline (PBS, Wako) and detached from the dish bottom using PBS based 0.025% trypsin and ethylenediaminetetraacetic acid (EDTA). The cell suspension was centrifuged at 1000 rpm for 5 min and re-suspended with fresh αMEM medium to form a concentration of 1×106 cells per ml. They were settled in a CO_2 incubator until used [12]. 4-Cytoplasmic estrogen receptor positive (ER+), malignant breast cancer MCF-7 (human, 69 year old Caucasian woman, adenocarcinoma) cells were used (Figure 1). The cells were cultured in a mixture media of 90% RPMI 1640, 10% FBS serum (ATTC, Manassas, VA), and 1% Penicillin/Streptomysin (Invitrogen, Carlsbad, CA.). The cells grow in an incubator at 37 o*C* with 5% CO2 [13]. 5-Human breast cancer cell line MCF-7 and MDA-MB- 231 were gifts from Prof. Jiangzhong Xi of the Department of Biomedical Enegineering in Peking University. Both cell lines were cultured in Dulbecco's modified Eagle's medium (ATCC) with 10% FBS (Sijiqing, Hangzhou, China) and 1% penicillin-streptomycin supplemented. Cells were maintained in the atmosphere of 5% CO2 at 37□ [14]. 7-Human womb neck cancer cell line, HeLa S3 cells (ATCC CCL-2.2) were maintained in alpha modified Eagle's α minimum essential medium (αMEM; GIBCO) containing 10% fetal bovine serum (FBS; GIBCO) and antibiotics at 37 ºC in a humidified, 5% CO2 incubator. The cultured cells sticking to the dish (100 mm; FALCON) bottom were washed twice using phosphate buffered saline (PBS) and detached from the dish using PBS based 2.5% trypsin-EDTA (Ethylenediaminetetracetic Acid). The cell suspension was centrifuged at 1000 rpm for 5 min and re-suspended with fresh αMEM medium to be 1×10^6 cells per ml. They were settled in a CO2 incubator until they were used [15]. In one report author also tested the effect of nsPEFs in vivo, after tumor formation in 8 week old C57B1/6 mice. Mice were vaccinated subcutaneously in both flanks (one side for treatment and the other side as control) with 5×10 Fibrosarcoma B10.2 cells in 0.1ml Hanks Balanced Salt Solution without Ca and Mg (HBSSw/o). Tumor masses about 5-10 mm in diameter formed. Using needle electrodes, the tumors were exposed to nsPEF *in vivo.*

2.4 Experimental Method

Electrodes made of stainless steel, connected to pulse generator through coaxial cable and Controlled pulsing sequence were imposed to cell culture in sterilized cuvette for examine in vitro or directly in vivo. Using voltage probe pulse was monitored. Pulses were applied to culture for required time. To observe the voltage waveform a voltage divider is also used in between load and source.

2.5 Testing of Apoptosis

Observing morphological changes in cell destruction of cells can be examined. There are various methods to observe it. Cells in which apoptosis is induced and fragmentation of DNA occurs, in other words caspases activated cells are stained by some staining chamicals, like Trypan blue, propidium iodide, Acridine Orange/Ethidium Bromide (AO/EB) etc and stained cells are observed by flocytometer. Apoptosis is

also determined by Annexin-V-FITC binding to phosphatidylserine (exists on plasma membrane) in the cells which are exposed by nsPEFs are studied by flocytometer. Scanning Electron Microscope (SEM) is also used for observation of dead cells. Observing release of intracellular calcium stored in endoplasmic reticulam and mitocondriya, cell destruction cand be examined. Calcium release by mitocondriya is considered as an begining of apoptosis event. Diaminobenzidine (DAB) can also be used for staining the apoptotic cells, and apoptotic nuclei become dark brown in color. Mostly used method is observing apoptosis using Propidium Iodide (PI) stain, and flocytometer to examine apoptosis. Weight of tumor is also be observed in vivo for the confirmation of optimistic result of treatment with nsPEFs.

Using a fluorescent molecular probe Propidium Iodide (PI), dead cells were identified, in population, which intercalates with DNA and fluoresces in red. Flow cytometer was for the inspection of apoptosis and necrosis (cell death by injury) of cells. It is capable examining both quantitavely as well as qualitatively. Flow cytometer is capable of fast testing. Main feature of apoptosis is fission of the nucleus, which can be observed by change in cell structure and in an increasing of DNA debris level in sample of cell, which is a method of measuring apoptosis. The method also needs cell samples to get stained with the use of DNA specific dye to detect the morphological change in cell nucleus. Annexin V and Propidium Iodide (PI), two lipophilic cationic dyes, were selected to change the color of mitochondria. Annexin V is used to bring out apoptotic cells. The Annexin V has an advantage to observe the phosphotidylserine, when the serine is transposed from the inside to outside of cell membrane. There are also numerous advantages of Annexin V. Annexin V can be used in live cells, and at a relatively early stage of apoptosis. PI is the commonly used dye for cell cycle analysis. PI goes into the groove of double stranded DNA and then colors the cell nuclei. In some reports diaminobenzidine was also used which causes apoptotic nuclei to become dark brown in color.

3.0 RESULTS AND DISCUSSION

Considering different published reports optimistic results have been found in vivo as well as in vitro. By analyzing different reports it has been confirmed, as pulse duration increases, the poration effect also increases and also shorter pulses causes a little effects on the plasma membrane. If electric field intensity is high enough it modifies intracellular structures sufficiently so induction of apoptosis occur in cells. The variation of temperature remains within the tolerance range of cells so the inhibitory effects were not due to the thermal effects of nsPEF rather than radiation therapy where temperature rise is more.

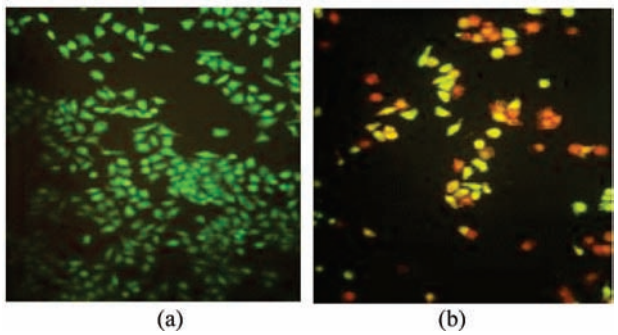

FIG. 2. RESULTS OBSERVED AFTER AO/EB STAINING. (A)A CONTROL GROUP B (B) TREATMENT GROUP

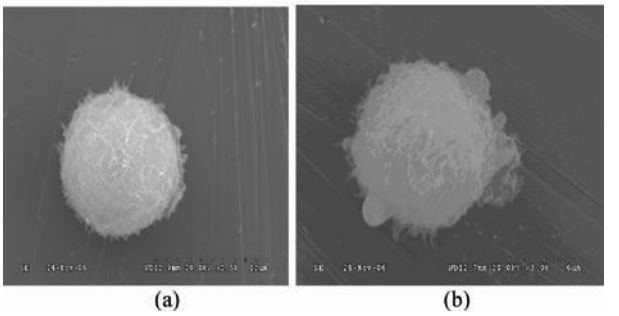

FIG. 3. SKOV3 CANCER APOPTOSIS OBSERVED UNDER SCANNING ELECTRON MICROSCOPE (SEM). (A) UNTREATED CELL (B) TREATED CELL

After nsPEF treatment few hours later, using Annexin V-FITC/PI, flow cytometry is used to measure the rate of early apoptosis and the total rate of late apoptosis and necrosis. It has been found, the servival of cell depends on amplitude as well as number of pulses. At electric field

25kV/cm and 60 pulses number, and 70ns pulse period, exposed HeLa S3 cells were found dead [12]. It has been also indicated that pulses of low intensity like 200 V/cm, duration of millisecond can be used to cell apoptosis induction in the MCF-7 cancer cells, compared to the conventional high intensity pulses in high numbers. Shows that very low doses of chemo drugs can be used along with pulse power technology with very less chances of side effect, those results are very encouraging for practical application of this technique to clinics. Patients would have another treatment modality with lower cost and fewer side effects, as the indication by the trials in clinics [13]. Using human eosinophils, It also has been pointed out that very short duration pulse with high electric field, without reaching to charging time of the plasma membrane subcellular vesicular membranes get breached [5]. Process of apoptosis by nsPEFs also has been explained in SKOV3 Cancer Cell (ovarian carcinoma cells) as, considering bioelectricity characteristics of each part, a cell can be esteemed as conductive cytoplasm and organelle (resistive equivalence approximately), surrounded by dielectric outer and inner membrane (capacitive equivalence approximately). Considering the equivalent capacitance of cell membrane, nsPEF penetrates to cell interior because of having higher frequency components in it, leading to a series of functional changes in mitochondria, endoplasmic reticulum, nucleolus and cell signal transfer (e.g. the activation of intracellular calcium stores). This may be one of the bioelectric mechanisms of how ns PEF affects SKOV3 cancer cells and then induces the cell apoptosis and thus cells in tumer/ cancer diminished and tumer/cancer get reduced in size [10].

Below are the few figures shows that optimistic result is found by the nsPEFs treatment. Photograph below shows changes in cell structure and density before and after the treatment. Untreated cells ane known as control group as they are controlled in a way such that they didnot die due to bacterial infection. Trated cells are compared with control group and difference is observed [11].

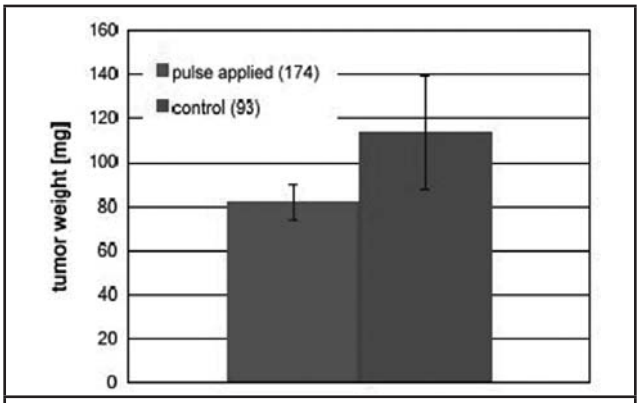

FIG. 4. COMPARISON OF THE TUMOR WEIGHT AFTER NSPEF APPLICATION TUMOR CELL AND CONTROLS CELL. THE CHARGING VOLTAGE OF THE PULSED POWER GENERATOR AND THE NUMBER OF PULSES APPLIED VARIED BETWEEN 2.61 KV AND 3.29 KV AND BETWEEN 1500 AND 15000 PULSES, RESPECTIVELY [7].

4.0 CONCLUSION

Experiments have gone through different types of cancer in mammalian. Experiments have been done in vivo and in vitro. By analyzing various reports is has been found that pulsed electric field of ms to few μs can cause electroporation while nano second pulsed electric field attacks on intracellular. Also in papers analyzed, critical limits of pulse duration, voltage level and number of treatment were not clearly defined. Therefore it required more experimental results to use the technology in practical life. Positive result has been found although, in case of human adenocarcinoma breast cancer [13]. Mostly results have been taken by growing tumer in mice in vivo or in vitro. In few reports, positive results have been demonstrated by cell modeling. Using COMSOL software, Model of cells was electrical and approximate behavior have been demonstrated [16]. Mathematical modeling also has been used for increased temperature when tumer was treated with nsPEF, although gives pessimistic result [17].

5.0 FUTURE SCOPES

Electroporation may be very useful phenomenon, as using electroporation drugs, which induced apoptosis, can be inserted across plasma membrane so that electrochemotharapy can be cheaper as

then there will not be nano second pulse treatment would be required and apoptosis can be achieved at ms or μs pulse ,less in numbers. This would be future of cancer treatment. Also pico second (sub nano pulsed electric field of high voltage will much more effective to induce apoptosis in cells. It would be challenging to design such pulse power generator because of difficulties associated with practical passive and active elements used in pulse generator. The experiment using nanosecond pulse electric fields (nsPEF) on solid tumors performed on embryonated chick assay. Confirming, the weight difference after, treatment using nsPEF. The effects of charging voltage and number of applied pulses were, confirming The difference of tumor weight between more and less than 2.85 kV significantly, However, as for as considering number of applied pulses, the significant difference did not confirmed. Therefore necessities are to investigate the number of applied pulses less than 1500 shots and more than 15000 shots [8].

Therefore areas where further work can be done are summerised as It is very costly to generate short pulses having range of few nanoseconds and picosecond of high voltage in this area further research is required also. Also to generate pulses having continuously varying amplitude and variable pulse duration is very difficult although discrete varying amplitude and variable pulse duration is easy but it is costly too. Mostly experiments had done in vitro rather than in vivo therefore it is required deep analysis for the feasibility for this type of treatment in vivo. Electrode design for electrical discharge in human body that could be practical for cancer treatment is also challenging. Also exact critical field value and pulse duration for electroporation and for apoptosis induction is also unknown. These are the some important areas where further work can be carried out for the treatment to be practical for goodness of society and advancement of medical science.

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