Effective Proliferation Control of Human Cancer Cells using Electrical Pulses

Raji Sundararajan, Funian Xiao, Therese Salameh, Lisa M. Reece, James F. Leary, Kevin Otto, Ignacio G. Camarillo
Purdue University, West Lafayette, IN 47907, USA

and **Luca G. Campana**
Melanoma and Sarcoma Unit, Instituto Oncologico Veneto (IOV)
Dept. of Oncological and Surgical Sciences - University of Padova
Via Gattamelata, 64 35128 - Padova (Italy)

ABSTRACT

Since current cancer therapies do not work effectively for all patients, there is a pressing need for alternate, affordable, yet effective treatments for inoperable and chemo-resistant tumors. Presented in this paper are our investigations on the application of short duration (milli and micro seconds) electrical voltage pulses for the treatment of breast carcinoma. For this purpose, electroporation, a pulse power technology, was evaluated for its effect upon the viability and proliferation of human breast cancer cells using a number of voltages and pulse durations. The results indicate the promising potential of electrical pulses to effectively and economically enhance drug transport across the normally impermeable plasma cell membranes and to reduce proliferation.

Index Terms — **Electroporation, MCF-7 breast cancer cell line, flow cytometry, western blot, impedance spectroscopy, proliferation control.**

1 INTRODUCTION

THERE is a pressing need for alternative physical therapies for unresponsive cancers to the conventional modalities of treatments including surgery, chemotherapy, radiotherapy, and immunotherapy. The poor response rates (Table 1 [1]) of many commonly prescribed breast cancer drugs and the death of a woman every 13 minutes in the US and over 400,000 worldwide necessitates this. Once a disease of the affluent industrialized western world, now cancer is everywhere [2]. It is estimated that 12.7 million cases and 7.6 Million deaths have occurred worldwide in 2008 and this number is only increasing [3]. Thus patients with in-operable, recurrent, chemo-resistant tumors need affordable, alternative treatments.

Table 1. Breast cancer drug response rates [1].

Drug	Response Rate
Paclitaxel	17% to 54%
Tamoxifen	21% to 41%
Doxorubicin	25% to 40%
Docetaxel	18% to 68%
Trastuzumab	10% to 20%

Electroporation or Electropermeabilization (EP) is a viable physical technique wherein high intensity, short duration pulses are applied to temporarily open up pores in the plasma membrane of cells, which otherwise are impermeable to allow transport of therapeutic materials including drugs, antibodies and genes (DNA). Since the application of pulses is only for a very short duration, the cell membranes eventually reseal and confine the drug molecules to act within the cell. A low dose of the chemo drug is injected intratumorally or intravenously, a few minutes before the application of the pulses.

When used to deliver chemo drugs, this technique is called Electro-Chemo-Therapy (ECT). Its success has been attested by the various clinical trails and ongoing treatments for extreme cases of melanomas, sarcomas, and other skin cancers including head and neck cancers [4-7]. When surgery, radio and chemotherapies didn't work, patients suffering from chest wall breast carcinoma benefitted using ECT [6, 7]. Thus, electrical pulse-mediated drug delivery is an attractive option where conventional treatments do not serve the patients.

Towards this end, we intend to explore the efficacy of this technique using classical FDA approved breast cancer drugs and low voltage long duration pulses along with those used in the clinical trials. The use of breast cancer chemotherapeutics for electroporation is novel as these drugs have not been studied yet. The most commonly used chemo drug in electroporation applications is bleomycin (occasionally, cisplatin) [6, 7]. Hence it is of practical interest to extend this technique to other primary breast cancer drugs.

Since the various pulse parameters, intensity of electric field, duration, number of pulses and interval between them, depend on the type of molecules, as described above, (equations, (1-6)), there is a need to test those pulse parameters.

Manuscript received on 28 November 2011, in final form 8 June 2012.

Electroporation is a physical process of generating pores in cell membrane [8-10]. During electroporation, drug molecules can enter the cancer cells in various ways, such as diffusion, electro-osmotic, and colloid-osmotic flow [11]. The magnitude of membrane potential can be defined [12] by Equation (1),

$$V = 1.5\,ER \quad (1)$$

where V is voltage induced on the cell membrane, E is electric field intensity applied, and R is the radius of cancer cell. The equation can be modified as,

$$E = 4V/(3D) \quad (2)$$

where D is the diameter of cancer cell. Thus, Equation (2) indicates that the E field intensity for a given potential V is inversely proportional to the diameter of cell. It also depends on the tissue and organ [13]. Hence, it is necessary to optimize the electroporation parameters for each cell type.

The flow of light and small drug molecules can be described by using the Fick equation (3) [14], for the transfer of drug molecules D, into the electroporated cancer cell of radius, Rc:

$$\phi(D) = 2\pi Rc^2 P_D \Delta D\, X\,(N,t_i)\,(1 - E_p/E)\, e^{(-f(N,\,ti)t)} \quad (3)$$

where $\Phi(D)$ is the flow at time t after N pulses of duration, t_i is the interval between two pulses, P_D is the permeability coefficient of drug across the electroporated membrane, ΔD is the concentration gradient of drug across the membrane. E_p is the threshold electric field for the membrane to break and E is the applied field strength. Equation (3) has its limitations, since it was derived under two assumptions. First, the drug molecules are not charged. Second, there is no resting potential difference across the cancer cell.

The Gouy Chapman [15] relationship between the drug molecule concentration (D) in the bulk media and that on the surface of the cell membrane is shown in Equation (4):

$$D_{\text{int}\,erface} = D_{bulk} e^{(\frac{-ze\Psi_o}{kT})} \quad (4)$$

where z is the number of charges on the molecule, e is the charge of an electron. k is the Boltzman constant, T is the temperature, Ψo is the membrane surface potential. Moreover, the cell membrane potential difference controls the extent of the diffusion across the cell membrane. The Nernst-Planck relationship can be used for presenting an electro-diffusive component Φ_E to the flux, which is given by:

$$\Phi_E(D) = -(P_D D \Delta\Psi F)/RT \quad (5)$$

where $\Delta\Psi$ is a cell membrane surface potential difference, F is the Faraday constant and R is the gas constant. Equation (3) can be modified by putting Equation (4) and Equation (5) together for ΔD, as

$$\Phi(D) = 2\pi R_C^2 P_D D_{Bulk} e^{(\frac{-ze\Psi_o}{kT} - \frac{\Delta\Psi F}{RT})} X(N,t_i)(1 - \frac{E_P}{E}) e^{(-f(N,t_i)t)} \quad (6)$$

Equation (6) shows the relationship between the electric field parameters and the molecular drug flux into the cell. Under the assumption that the more drug molecules enter the cell, the drug influx describes the reduction in cell viability. Thus, the electrical pulses and electrical field parameter have to be optimized in order to optimize pore formation in cell membrane without affecting cell's functionality or even killing the cell.

Low voltage, long duration pulses are studied in this study as they are preferred for use on humans and animals [16] to high voltage pulses and also it is simpler to design and build a low voltage pulse generator than a high voltage pulse generator. Towards that goal, the pulse parameters were chosen and studied.

2 MATERIALS AND METHODS

2.1 MCF-7 HUMAN ADENOCARCINOMA CELLS

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 ^oC with 5% CO2.

For electroporation, cells were washed twice with 1xPBS whose pH was adjusted to 7.4, and left in serum-free 199 medium (Invitrogen) for 24 hours. Cells were dissociated from the incubation flask with 0.25% trypsin/EDTA (ATCC) solution. A hemocytometer was used to obtain a final concentration of 1x 10^6 cells/mL. Aliquots of 750 μL in 0.4 cm cuvettes were used for electroporation by adding RPMI 1640 medium with 10% charcoal stripped fetal bovine serum.

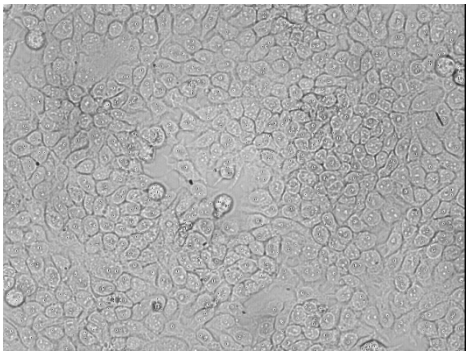


Figure 1. Human Estrogen Receptor positive breast Adeno-carcinoma cell line.

2.2 CHEMO AND HORMONE THERAPY DRUGS

FDA approved, commercial chemo and hormone drugs for breast cancer treatment including Paclitaxel (Taxol), and Bleomycin (Bleo), and Tamoxifen (Tam) were studied. They were used at very low doses compared to that normally used in clinical chemotherapy. All these have life time cumulative dose limits and side effects [17], including fever, chills, skin reactions, hair loss, nausea, vomiting, diarrhea, and muscle pain, alopecia, gastrointestinal issues, etc., thus, ideal candidates for EP.

2.2.1 PACLITAXEL (TAXOL) CHEMO DRUG

Taxol is a FDA approved chemo drug for advanced breast cancer treatment. This was first discovered from the bark of the slow-growing Pacific Yew tree and has proven to be

highly effective in treating women with advanced breast cancer [6]. It inhibits disassembly and reorganization of the microtubule structures necessary for cell division. Thus, it is a cellular inhibitor type of anticancer drug. Its chemical structure is shown in Fig. 2a. The major feature of Paclitaxel as an anticancer drug is that it interacts with tubulin synthesis in cancer cells without affecting any normal cells. Although its details are not fully understood, the mechanism for the anticancer activity of Paclitaxel appears to be related to the alteration of the polymerization equilibrium of tubulin [15]. The compounds of Paclitaxel are small size organic molecules, and their efficacy extends much deeper than a generic resemblance in shape. In addition, these compounds change in their electronic structures, orientation, and isomerization of its tail and are characterized by a rather large dipole. These modifications not only increase the pharmacological activity of Paclitaxel, but also enhance its peculiar features. These properties had been proven by the fact that the binding between Paclitaxel and tubulin is competitive and mutually exclusive [18]. They also suggest that this molecule can be used for an adaptive link to a neighboring docking site on the same or different tubulin dimer. Common side effects include low white and red blood cell counts, weakness, hair loss, fatigue, nausea, vomiting, diarrhea, and muscle pain, as well as numbness, tingling, and burning sensations in the arms and legs. Thus, it is an ideal candidate for ECT, as a very low dose (9 nM) could be used and hence reduces side effects and cost.

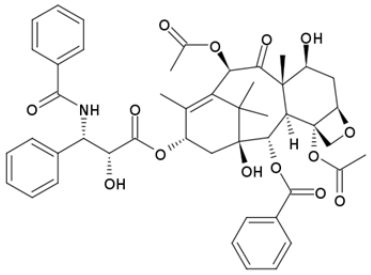


Figure 2a. Chemical/Atomic Structure of Paclitaxel.

2.2.2 BLEOMYCIN CHEMO DRUG

Bleomycin is another FDA approved cytotoxic drug for anticancer chemotherapy for the treatments of squamous cell carcinomas, testicular carcinoma, melanomas, sarcomas, and lymphomas [4, 5]. It is a DNA targeting, cytotoxic, antitumor, antibiotic chemo drug. Its structure is shown in Fig. 2b. It binds oxygen and redox active transition metal ions, such as Fe^{2}, Cu^{2+}, Zn^{2+} and so on. When Bleomycin interacts with a nucleic acid, it results in the generation of a basic site, a base propenal, and a break in the DNA strand. The main function of Bleomycin as an anticancer drug is the cleavage of DNA, because it cuts DNA at the level of the GC base pairs [19, 20]. The mechanism of action of Bleomycin is that the bithiazole and the terminal amine parts bind stably with the DNA. This results in one double-strand for eight single-strand DNA breaks on average. Thus, there are more double-strand DNA breaks will appear more than usual under normal situation. In addition, Bleomycin has also specificity of the cleavage on chromatin. Bleomycin has accessibility into the sequence of DNA, which is consisted by two consecutive nucleosomes. In other words, the molecules between single-strand and double strand stretches can be bound and broken by Bleomycin. Bleomycin is considered as a cytotoxic anticancer drug due to DNA double-stand cleavages and the loss of chromosome fragments [19]. Common side effects include fever, chills, skin reactions, hair loss, alopecia, and gastrointestinal issues [17].

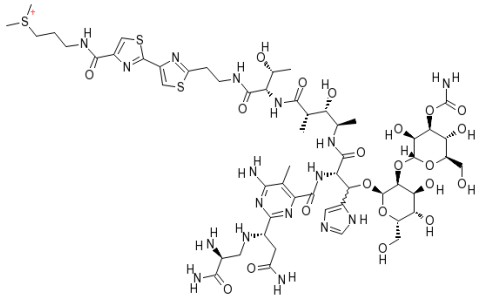


Figure 2b. Chemical/Atomic Structure of Bleomycin.

2.2.3 TAMOXIFEN

Tamoxifen is one of the most effective endocrine agents for treatment of breast cancer [21]. Its structure is shown in Fig. 2c. It is a selective estrogen receptor (ER) modulator (SERM) with tissue-specific activity. It first binds to ER and forms a complex and this interacts with the DNA. The ER/tamoxifen complex recruits other proteins known as co-repressors to stop genes being switched on by estrogen. Some of these proteins include NCoR and SMRT [22]. Tamoxifen's function can be regulated by a number of different variables including growth factors [23]. It can block growth factor proteins such as ErbB2/HER2 [24] as high levels of ERBB2 have been shown to occur in Tamoxifen-resistant cancers [25]. Tamoxifen seems to require PAX2 protein for its full anticancer effect [17]. In the presence of high PAX2 expression, the tamoxifen/estrogen receptor complex is able to suppress the expression of the pro-proliferative ERBB2 protein.

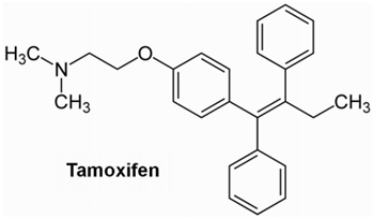


Figure 2c. Chemical/Atomic Structure of Tamoxifen.

2.3 ELECTROPORATION TECHNIQUE

For efficient electroporation, two conditions have to be satisfied. First, a sufficient amount of chemotherapeutic drug must be present in the targeted tissue/tumor when the pulses are applied. Second, electric pulses have to be of appropriate magnitude and duration to create reversible (transient) pores in the cell plasma membranes. Thus, it is critical to choose appropriate electrical parameters to achieve pore formation in the cell membrane without cell death and a pulse generator

(pulser) to apply the required pulses. In this study, a BTX ECM830 square wave electroporator (Genetronics, Inc., San Diego, CA) was used. This generates square wave pulses under both low voltage and high voltage modes. The voltage changes from 5 to 500 V with 1V resolution under low voltage mode, and from 505 V to 3000 V with 5V resolution under high voltage mode. The pulse width ranges from 10 ms to 100 ms under low voltage mode, and from 10 μs to 600 μs under high voltage mode. A cuvette holder holds cuvette with cells and is connected to the generator.

Eight, 1200 V/cm, 100 μs pulses were used in the skin cancer and chest wall breast carcinoma clinical trials [4-6]. In our study, we tested both low voltage (200-500 V/cm), long duration (milliseconds, ms) and high voltage (1200 V/cm), short duration (microseconds, μs). Eight sequential pulses with 1 Hz frequency [5] were used in all the cases to explore the electroporation efficacy in this study.

2.4 CHARACTERIZATION ASSAYS

MCF-7 cancer cells were treated with various drugs and a number of electrical and dose parameters. Treated cells were cultured at 37 ºC, 5% CO_2 incubator for 24 h before analyzing using various assays, such as cell viability and growth assay, Fluorescence-Activated Cell Sorter (FACS) assay, western blot, and electrical impedance spectroscopy for the effectiveness of the applied pulses.

2.4.1 CELL VIABILITY AND GROWTH ASSAY

After electroporation cells must remain in cuvettes for 30 minutes before being transferred to well plates for incubation. After 24 h, media and dead, unadhered cells were removed from the wells. Live, adhered, cells were dissociated from the incubation flask with 0.25% trypsin/EDTA (ATCC) solution. Live cells were counted and recorded with the Cellometer Auto T4 from Nexcelom Bioscience, LLC., using Trypan Blue assay.

***Preparation of cells*:** after electroporation and incubation, cells suspended in media were stained with a 1:1 concentration of Trypan Blue. The dead cells stained blue so they could be distinguished from the clear, live cells and were counted using the Cellometer.

2.4.2 FLOW CYTOMETRY OR FLUORESCENCE-ACTIVATED CELL SORTER (FACS) ASSAY

Flow cytometry is a technique used to count, examine, and sort microscopic particles suspended in fluid, including dead cells, both qualitatively and quantitatively [26]. Thus, it is used widely for the two modes of cell death analysis, apoptosis and necrosis. While necrosis refers to a simple situation that the cell is dead, apoptosis is a more complex process in cell death. Necrosis becomes a response when a cell is injured. There are many processes that lead to necrosis, including loss of ATP in cytoplasm, loss of cell membrane integrity, and so on. When the cell size is reduced, the cell is considered as dead by apoptosis. Other processes of cells, including the loss of membrane potential, a decrease in cell size, the loss of mitochondrial function, altered cellular redox status, and so on, is also accounted as apoptosis. Although some of necrosis progresses can eventually occur in the late stages of apoptosis, the loss of plasma membrane function in the early stage becomes a characteristic feature of apoptosis. The occurrence of apoptosis in cell depends on the type of cell and the trigger of the apoptosis. Fragmentation of the nucleus is the main feature of apoptosis, which can be evaluated by morphology and observed in an increased level of DNA debris in cell samples. Thus, the level of DNA debris can be taken as a method to measure apoptosis, and hence the efficacy of ECT. The method needs cell samples to be stained using a DNA specific dye in order to detect the morphologic changes in nucleus. In this study, two lipophilic cationic dyes, Annexin V and Propidium Iodide (PI) were selected to stain mitochondria. Annexin V is used to reveal apoptotic cells. The Annexin V has an advantage to observe the phosphotidyl serine, 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 most common dye for cell cycle analysis. Typically, PI goes into the groove of double stranded DNA and then colors the cell nuclei.

***Preparation of cells*:** The electroporated cells were treated using fluorescence reagents, Annexin V Incubation Reagent (AVIR), and a binding buffer. The AVIR was prepared using various chemicals and was kept in ice in the dark after preparation. The binding buffer was prepared by diluting 50 μL of 10x binding buffer into 450 μL dH_2O, and then kept in ice. The cells were washed twice with 500 μL cold 1x PBS and were resuspended using AVIR and incubated for 15 minutes in the dark at room temperature. The binding buffer was added and used for flow cytometry using Cell Lab Quanta^{TM} SC flow cytometer with 488 nm Laser (Beckman Coulter) within one hour to get good data with maximal signal.

2.4.3 WESTERN BLOT

Western blot is a commonly used analytical technique to identify the specific proteins in the given sample using electrophoresis [27, 28]. In this study, total protein concentrations were determined using a p38MAPK protein assay to study the combined effect of electroporation and Taxol chemo drug.

***Preparation of cell proteins*:** Treated cells were washed 3x in ice-cold PBS. Whole cell lysate extracts were prepared by lysing cells in 50 μL lysis buffer (20 mmol/L Tris-HCl (pH 7.5), 150 mM/L NaCl, 1 mM/L EGTA, 1% Triton X-100 and complete protease and phosphatase inhibitor cocktails (Sigma)). Cell debris was removed by centrifugation at 14000xg for 20 minutes at 4°C and then cell lysate were sonicated (5x15s pulse). Amount of protein in each sample was determined using the BCA protein assay kit (Pierce; Rockford, IL).

20 μg of protein lysate was boiled for 5 minutes and subjected to electrophoresis in denaturing 10% SDS-PAGE. Membranes were probed for p38 MAPK (1:1000; Epitomics; Burlingame, California) overnight at 4 °C. Horseradish-peroxidase conjugated anti-rabbit IgG was used as a secondary (1:5000 for 4h at RT). The membrane was developed using an ECL reagent.

The membrane was then stripped using 0.2 M NaOH for 20 minutes at RT. The stripped blot was reprobed with β-actin (1:10000) as a loading control and developed using horseradish-peroxidase conjugated anti-mouse IgG (1:10000 for 1h at RT).

2.4.4 ELECTRICAL IMPEDANCE SPECTROSCOPY

Passive electrical properties, such as the complex impedance of biological materials, including human cancer cells have always been of special interest for their applications in therapeutic diagnosis and therapy. The impedance of biological cells is characterized by a variety of frequency-dependent changes (dielectric dispersion) [12, 13]. These changes are due to the permittivity and conductivity (or resistivity) contributions of various relaxation processes that follow due to the variations in the external applied field.

Impedance spectroscopy (IS) is an ac measurement technique in which the ratio of voltage and current is measured over a range of frequencies. It is a non-invasive, nondestructive test technique in which very little energy is dissipated by the system under test, leaving it virtually unaffected. This method is useful to characterize cellular changes quantitatively. It can be used as a method of identifying and following detectable cellular responses, in ex vivo, in vivo and in vitro [29, 30]. IS measures the electrical properties, i.e. the conductance (or resistance) and the reactance of any material, as a function of applied voltage frequency, as each polarizable entity within the tissue will exhibit its own characteristic response and thus a distribution of relative permittivities will give rise to a complex function of frequency of the form [31]:

$$\varepsilon^*(\omega) = \varepsilon_\infty + (\varepsilon_s - \varepsilon_\infty)/(1 + j\omega\tau) \qquad (7)$$

where ε_∞ is the high frequency permittivity at which the polarizable entities are unable to respond, ε_s is the low frequency permittivity where polarization is maximal, ω is the angular frequency, and τ is the characteristic relaxation time of the tissue under study. A dielectric dispersion is therefore associated with biological tissues [32] in which the relative permittivity decreases with increasing frequency. However, the displacement current, a function of the relative permittivity is proportional to the applied field frequency, and so these two opposing factors lead to a complicated frequency behavior. In general, three discrete regions of dispersion, alpha, beta, and gamma can be identified in biological tissues as depicted by Schwan [32].

This frequency-dependent relationship between impedance (z), conductivity (σ) and relative permittivity (εr) is given by the expression [33]:

$$Z = Z' + j\omega Z'' = 1 / (\sigma + j\omega\varepsilon_o\varepsilon_r) \qquad (8)$$

where Z is the total (complex) impedance, Z' and Z" are the real and imaginary components of Z respectively, ω is the angular frequency, and ε_o is the permittivity of free space. Both Z' and Z" were measured, from which the conductivity and relative permittivity can be calculated. It was assumed that the tissues have no or negligible inductive influence. Electropermeabilization affects the membrane resistivity. We investigated the impedance method as an approach to understanding the cell's enhanced conduction under control, drug only and electroporation conditions. For this, the AC impedance at room temperature was measured using a PGSTAT100 (Autolab) high voltage potentiostat/ galvanostat with a compliance voltage of 100V and a maximum current of 250 mA. It gives 40 data points for a frequency range from 0.5 Hz to 100 kHz.

Preparation of cells: The electroporated cells in the cuvette were connected to the potentiostat and a sine voltage of 25 mV amplitude was applied. The samples were scanned at 15 frequency points over the range of 46 Hz to 10,000 Hz. The data were acquired and impedance plots were plotted for analysis using a MATLAB code. The electrical impedance was displayed as Real Z (R, the resistive component in Ohm) and Imaginary Z (the capacitive reactance component in negative Ohm).

2.4.5 HUMAN CLINICAL TRIALS

The recurrence of breast cancer after a mastectomy is known as chest wall breast carcinoma. It may involve skin, muscle and fascia beneath the site of the original breast tumor, as well as lymph nodes. Chest wall recurrence can be treated with surgery, chemotherapy, and radiation. But there are inoperable cases and some do not respond to systemic chemotherapy [6]. For these cases, ECT is an alternative which works well for several patients [6, 7]. ECT is an effective drug delivery technique useful for treatment of cutaneous or subcutaneous cancers for patients with recurring, progressive, or inoperable nodules, refractory to the conventional anticancer therapies [6, 7]. It enhances the local toxicity of bleomycin, which is a non-permeant hydrophilic drug. It can be applied on externally accessible tumors of any histology and any part of the body. It is safe, simple, efficient and economical and gives consistent reproducible results. It also provides efficient palliation from painful, ulcerated, or bleeding tumors [5] and enhances quality of life for these patients, irrespective of the severity of the cases.

Eleven patients with chest wall recurrence from breast carcinoma were selected for this clinical trial, from the study in Italy by Campana et al [7]. These patients were selected per Standard Operating Procedure of the Electrochemotherapy (ESOPE) [34]. Briefly, the patients should have measurable tumor nodules suitable for electrode application. Exclusion criteria include serious lung, heart, or liver disease, epilepsy, short life expectancy (< 3 months), active infection, previous treatment with bleomycin to maximal dosage, and different anticancer therapies administered within 2 weeks before ECT. The only deviation from that protocol was the inclusion of nodules >3 cm. These patients had a total of 174 nodules of various sizes, ranging from <5 mm to >3 cm.

The patients were treated with local anesthesia and/or an addition of intravenous sedation was given as required in each case. Bleomycin was administered intravenously, intratumorally, or in a combination. Eight, 1200 V/cm, 100 µs pulses were administered within a few minutes, at a frequency of 5000 Hz (0.2 ms interval between pulses) using 4mm gap parallel array needle electrodes. Several applications were given when the tumor size was more than 4 mm to cover the entire tumor area. In addition, patients with head and neck cancers were also treated using the same pulse parameters. This high frequency was used to reduce the pain if any, as at this frequency, patients do not feel the pulsing.

3 RESULTS AND DISCUSSION

3.1 CELL VIABILITY AND GROWTH ASSAY

Cell viability results are shown in Figs. 3a and b for drugs, Taxol and Bleo, illustrating the efficacy of the electrical pulses in controlling/reducing the proliferation of the cancer cells using low and high voltage pulses at very small doses of the drugs. Table 2 shows the pulse parameters used for Taxol and Bleo. Eight pulses each of 200 V/cm, 40 ms and 1200 V/cm, 200 µs were used at one second interval (200 µs pulses were used in this study compared to 100 µs used in clinical trials, because, 100 µs was not sufficient to open pores with these drugs).

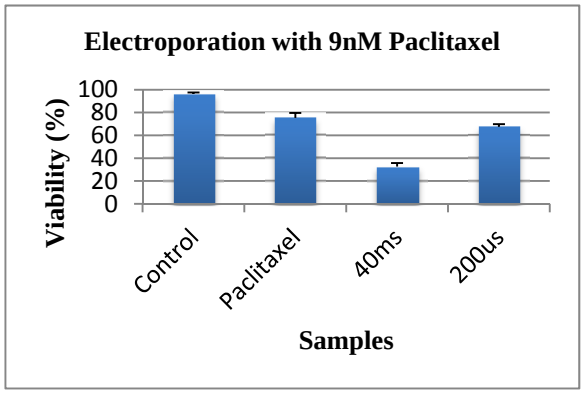


Figure 3a. Proliferation control using EP and Taxol at 9nM.

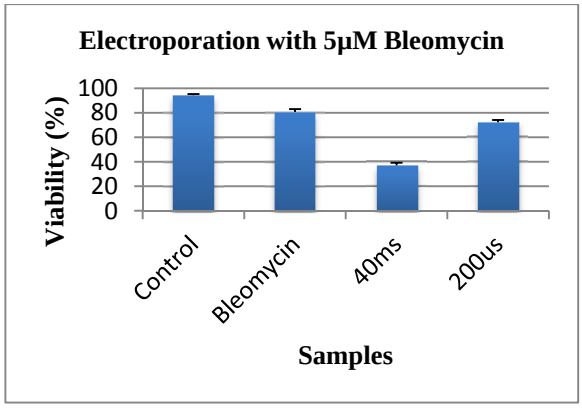


Figure 3b. Proliferation control using EP and Bleo at 5µM.

Figure 3a shows the results for delivering Paclitaxel, for the control (no treatment), drug only, and the two electroporated samples using 1200 V/cm, 200 µs and 200 V/cm, 40ms pulse parameters. The dose used was 9 nM. The viability of cells with drug only is reduced approximately 20% compared to the control. Cell viability of 9 nM Paclitaxel and 200 µs duration electrical pulse decreased to 65%. The same concentration Paclitaxel treated cells with 40 ms electroporated had a further 30% reduction compared to the 200 µs electroporated cells. Cell viability with Paclitaxel and 40 ms electroporation showed a reduction of more than 60% compared to the control and 40% compared to the Paclitaxel only. These results indicate the enhanced efficacy of the combination therapy with electrical pulses and the chemo drug at concentrations as low as 9 nM. Similar results were obtained for Bleo (Fig. 3b), but at a larger dose of 5 µM, indicating the high toxicity of Taxol, an ideal candidate for electrochemotherapy using reduced dosage.

Table 2. Samples and pulse parameters used for Taxol and Bleo Electroporatiom.

Sample	Electric field intensity (V/cm)	Pulse duration
Control (no treatment)	0	0
Drug control	0	0
EP-200 µs	1200	200 µs
EP-40 ms	200	40 ms

Figure 4 shows the viability of MCF-7 cells at varying pulse parameters using 5 µM Tamoxifen. Table 3 shows the micro- and millisecond pulses used to deliver Tamoxifen in the cells. Untreated samples were used as controls. Various treatment conditions as indicated in Figure 4 were studied.

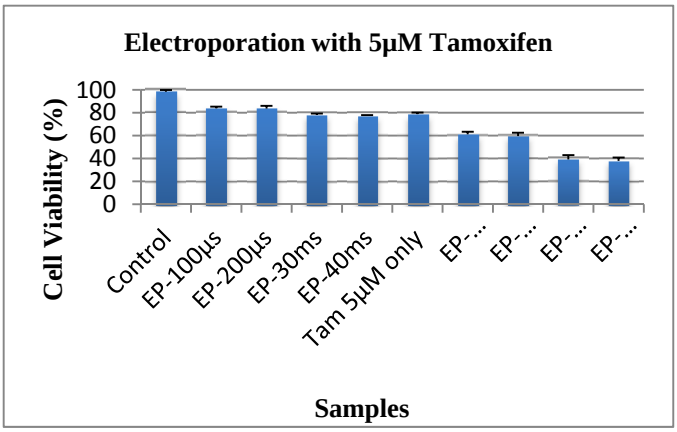


Figure 4. Proliferation control using EP and Taxol at 9nM.

Table 3. Samples and pulse parameters used for Tamoxifen electroporation.

Sample	Electric field intensity (V/cm)	Pulse Duration
EP-100 µs	1200	100 µs
EP-200 µs	1200	200 µs
EP-30 ms	200	30 ms
EP-40 ms	200	40 ms

The viability of the samples with 100 and 200µs pulse durations were reduced almost 15% compared to the control, while the others with millisecond pulse durations were reduced 20% compared to control. The viability of cells treated with 5 µM Tam only was reduced almost 20% compared with control and was similar to the viability of samples treated with only electroporation. The viability of MCF-7 cancer cells treated with 40 ms electroporation and 5 µM Tamoxifen is around 38% and the viability of cells treated with 30 ms electroporation and 5 µM Tamoxifen is 39%. The viability of both samples treated with microsecond pulses shows more than 50% reduction compared with control, almost 40% reduction compared with electroporation only and Tamoxifen only, and 20% compared with these samples treated with the millisecond electrical pulses and 5 µM Tamoxifen. The samples treated with millisecond electrical pulses and 5 µM Tamoxifen have similar viabilities, which are around 60%. The millisecond electroporated samples treated with 5 µM Tamoxifen have 30% less viabilities than control and around 20% less than electroporation only and Tamoxifen only.

Figure 5 shows the results of a yet another study where the live cell numbers and hence the viability of the MCF-7 cells after the electroporation in the presence of 1 µM concentration of Tam were determined. In this case, the pulses applied were 200 V/cm, at 10, 20, 30 and 40 ms, denoted by LP1, LP2,

LP3, and LP4 respectively. Untreated cells served as control. In this case, the 40 ms sample showed the least viability compared to the other reduced durations. This is expected due to the higher energy supplied with longer pulses, and hence more cells porated and a higher death rate.

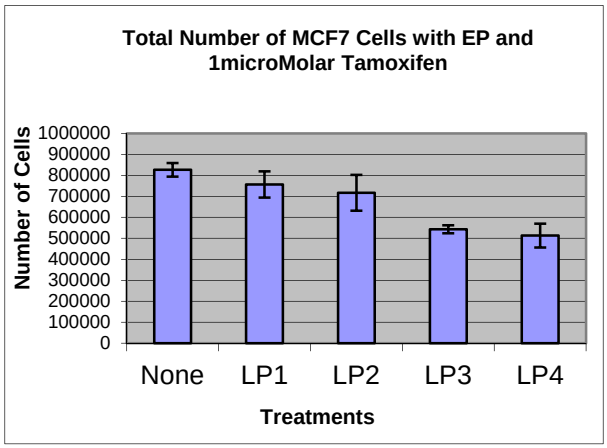


Figure 5. Viability using electroporation and 1 μM Tam. LP1, 2, 3, and 4 correspond to 200V/cm, 10, 20, 30, and 40ms pulses respectively – 8 pulses at one second interval. None is the control sample that was not treated.

3.2 Flow Cytometry

Figure 6 displays the results obtained using flow cytometry. There are four areas in each image showing the status of the cells, as alive (blue), early apoptotic (purple), apoptotic (brown), and dead (red). Two different deaths are shown in these figures, apoptotic (programmed cell death) and dead. It also indicates the various conditions of the cells by using two parameters, FL1 (green fluorescence) and FL3 (red fluorescence). FL1 displays the live cell data from Annexin V while FL3 displays the dead cell data from propidium iodide (PI). The peak emission wavelength for Alexa-488-Annexin V is 528 nm, while the peak emission wavelength for PI is 600 nm. Figure 6a shows the control sample. In this case, a large number of cancer cells are located in the live area. When these cells were treated with 5 μM Bleomycin only, more cancer cells were moved from live area to apoptotic and dead area, as illustrated in Figure 6b. Due to electroporation, more cells were dead compared to control, and moved from live and apoptotic areas to dead area. Especially compared between 40 ms and 200 μs electroporated samples with Bleomycin, more cells moved from apoptotic area to the dead area, as illustrated in Fig. 6(c). Figs 6(d-f) show the MCF-7 breast cancer cells treated by Paclitaxel without or with electroporation. The same pattern as in for Bleomycin was found in the case of Taxol too. Table IV shows quantitatively the percentage results of the breast cancer cells that were counted and computed using FACS for the four different areas, dead, early apoptotic, apoptotic, and viable breast cancer cells, of each case in Figure 6.

These results correlate previous research findings as it was reported that a number of anticancer drugs induce apoptosis in cancer cells [35]. Apoptosis occurs in most, if not all solid cancers. It is preferred to have apoptotic cell death, as opposed to necrosis, which involves inflammation, swelling of organelles, clumping of chromatic, and membrane disintegration. In contrast, apoptotic cells contain chromatin compacted into sharply delineated masses, and condensation of the cytoplasm occurs. No inflammatory reaction is noted due to apoptotic cell death. As seen in Figure 6, most MCF-7 cancer cells were located in the dead area after using electrical pulses to deliver anticancer drug. The results indicate that electrical pulses-mediated anticancer drug delivery enhanced the efficacy of curing breast cancer, because this technique can improve the permeability of the cell membrane in MCF-7 breast cancer cells.

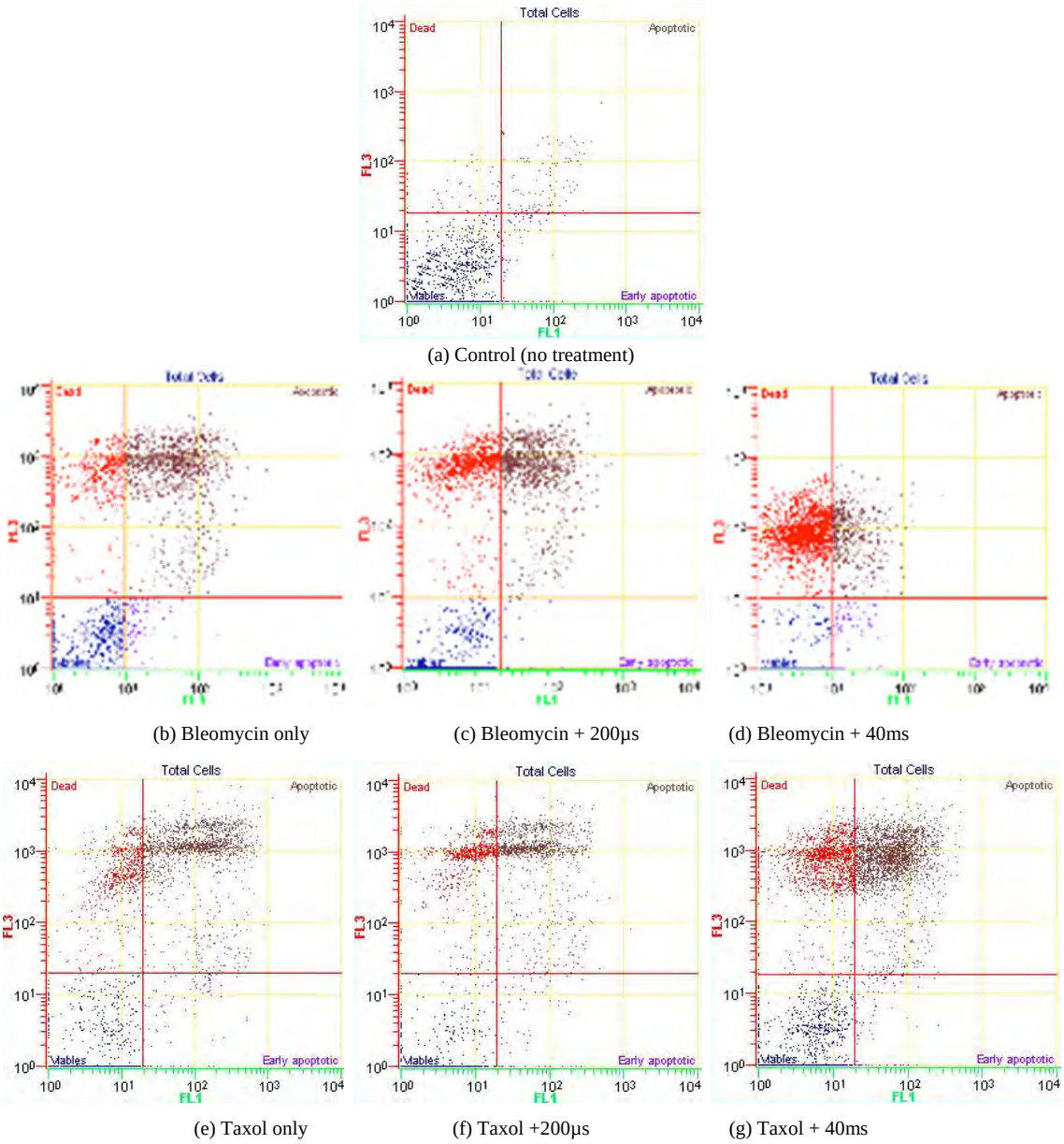
3.3 WESTERN BLOT

To examine the physiological response of applied pulses, the MCF-7 cells treated with Taxol and electroporation were studied for levels of protein, p38MAPK, because, with Taxol, there is an increased level of p38MAPK expression. As anticipated, our results showed increased p38MAPK expression with Taxol (Figures 7a and 7b). This agrees with the work of other researchers [36]**.** Compared to the control, MCF-7 cells treated with Taxol only shows an increased expression of p38MAPK. MCF-7 cells that were treated with Taxol and electroporated also showed increased p38MAPK expression compared to control. However, there is a reduction compared to Taxol only. This could be due to the increased cell death with the combination of drug and electrical pulses and the dead cells being washed away.

3.4 IMPEDANCE SPECTROSCOPY

Figure 8 displays the impedance spectrum illustrating the frequency dependent dielectric constant and conductivity of control sample of the MCF-7 human adenocarcinoma breast cancer cells over 50 to 10,000 Hz. The capacitive reactance (X_C), the imaginary component, represents dielectric constant which characterizes the ability of the cell to store electric charge, in addition to other parameters related to capacitance of the cell. The resistance (R), the real component, which is a measure of the conductivity is also shown, as the conductivity is simply a measure of the ability to transport charges with the field [37]. These two properties solely characterize the electrical characteristics of matter. The magnetic properties of the cells are not considered as they are virtually identical with those of free space.

These spectra display two unique features of biodielectric cells: a) the dielectric constant reaches very high values as the frequency decreases below 100 Hz, and b) the values change in two distinct steps at the measured frequency range, each of them of a clearly "dispersive" (frequency dependent) nature. These are the alpha (α) dispersion in the frequency range up to 1000 Hz and the beta (β) dispersion in the frequency range higher than 1,000 Hz. The beta dispersion is caused by the polarization of cellular membranes with the cytoplasmic and extracellular media serving as access paths for the charging currents. It is a Maxwell-Wagner relaxation effect. The alpha effect may have several causes, including the charging of intracellular structures connecting with the outer membrane, counter ion relaxation effects and frequency dependencies of membrane itself [37]. In addition, various fine structural contributions have been identified which broaden the tail of the beta-dispersion.These include relaxation of protein bound water, polarization of cellular organelles, such as nucleus and mitochondria, and dielectric relaxation of the proteins in the cells [38].



(a) Control (no treatment)

(b) Bleomycin only (c) Bleomycin + 200µs (d) Bleomycin + 40ms

(e) Taxol only (f) Taxol +200µs (g) Taxol + 40ms

Figure 6. Flow cytometry bivariate histograms of PI (FL3) vs. Alexa-488-AnnexinV (FL1) illustrating the status of cells at various conditions.

Figure 9 shows the impedance characteristics of the drug only samples, Figure 9a shows the impedance spectrograph for Paclitaxel and Figure 9b shows that of Bleomycin. They closely follow the control sample, both in magnitude and trend.

Figures 10 and 11 show respectively the impedance spectroscopy of the electroporated samples using 1200 V/cm, 200 µs, and 200 V/cm, 40 ms, with Taxol and Bleo. There is a dramatic decrease, up to 4 orders, in the magnitude of the impedance, both the real and the imaginary components in the case of elctroporated samples compared to control and drug only samples. This clearly indicates the enhanced increase in conduction or permeability of the cell membrane due to the application of pulses. The magnitudes were in the order of 10^7 for control and drug only samples, while they are in lower hundreds (10^2) for the electroporated samples with two different electrical pulse durations.

It can be seen that all these impedance plots follow α and β dispersions as well as a fractal power law with negative exponent (i.e. inverse power law) typical of bio dielectrics [9, 13, 39]. The variations in the negative power indices reflect the differences between electroporated and the control and drug only samples. The electroporated samples have a lower index of around -0.5, while the control and drug only samples have indices > 0.5. These results agree very well with the previous results reported by [12, 38].

3.5 HUMAN CLINICAL TRIALS

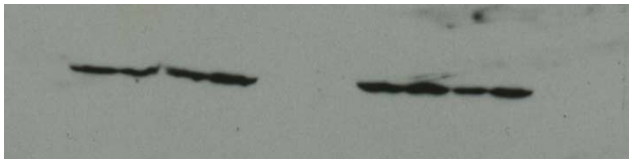
There was complete recovery of the chest wall breast carcinoma patient who did not respond to conventional chemotherapy. Figure 12 a shows before treatment and Fig. 12b shows the cure after just 7 days. Similar results were also

reported by Larkin, et al, from Ireland's Mercy University Hospital [6].

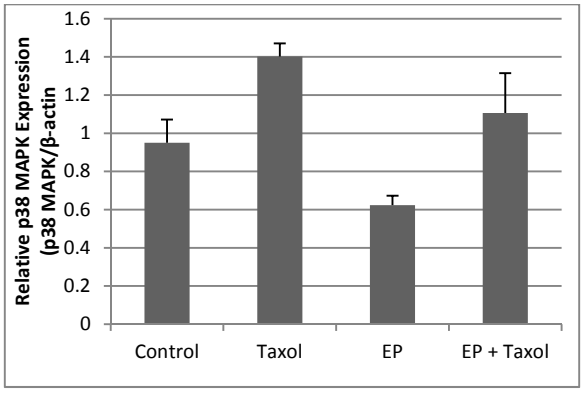
The head and neck cancer case is shown in Figure 13. Antitumor activity was observed in all tumor types.

Table 4. Status of the cells without Electroporation.

Item	% Status			
	Dead	Early Apoptotic	Apoptotic	Viable
Control	3.97	2.19	4.03	89.81
Bleomycin only	18.74	4.85	36.73	39.68
Bleomycin + 200 µs	28.93	1.07	43.38	26.62
Bleomycin + 40 ms	64.57	4.25	23.62	9.56
Taxol only	28.14	5.71	43.79	22.36
Taxol + 200 µs	39.48	2.67	42.15	15.70
Taxol + 40 ms	67.92	0.53	24.96	6.59



(a) Western Blot illustrating protein levels



(b) Western Blot illustrating protein levels quantitaively

Figure 7. Western Blot (a) llustrating higer protein level for EP+Taxol than control sample (b).

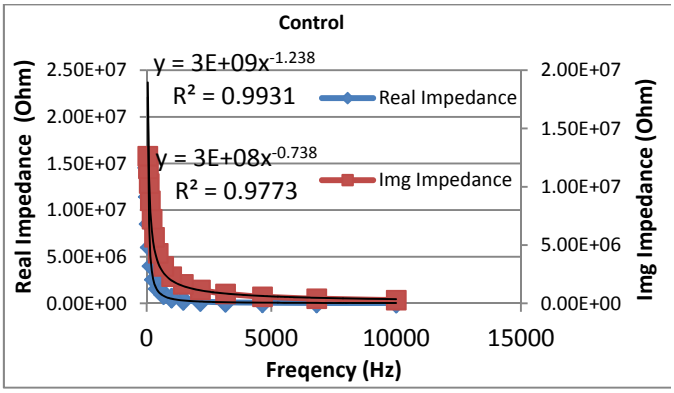


Figure 8. Impedance Spectrum of MCF-7 breast cancer cells -control (no treatment). Negative Img Z is shown.

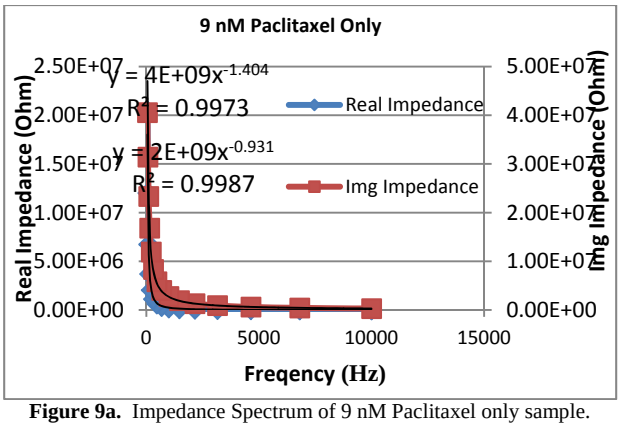


Figure 9a. Impedance Spectrum of 9 nM Paclitaxel only sample.

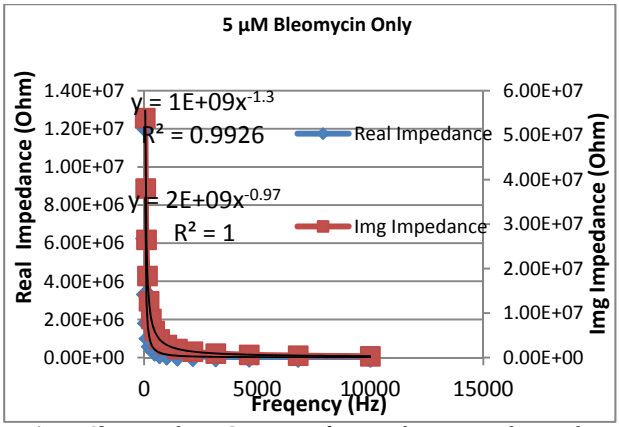


Figure 9b. Impedance Spectrum of 5 µM Bleomycin only sample.

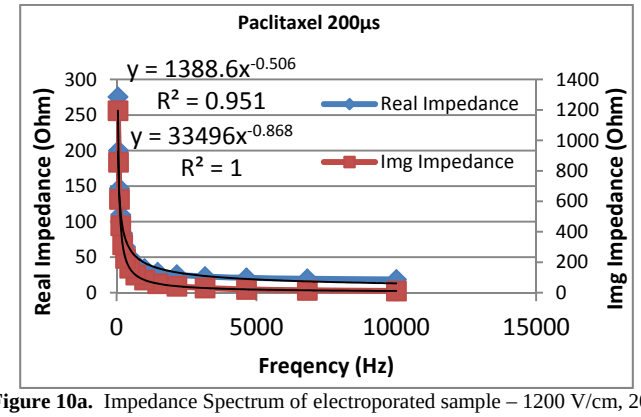


Figure 10a. Impedance Spectrum of electroporated sample – 1200 V/cm, 200 µs pulses using Paclitaxel.

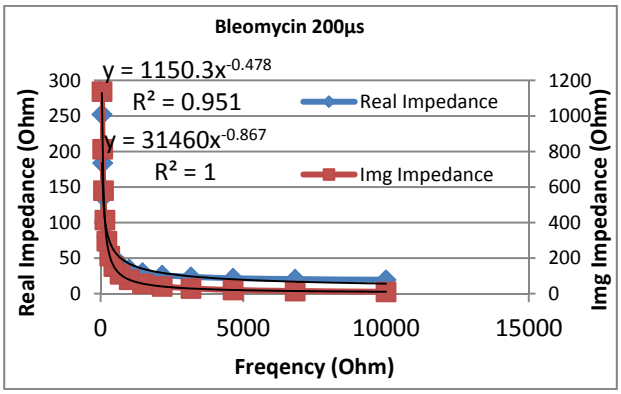


Figure 10b. Impedance Spectrum of electroporated sample – 1200 V/cm, 200 µs pulses using Bleomycin.

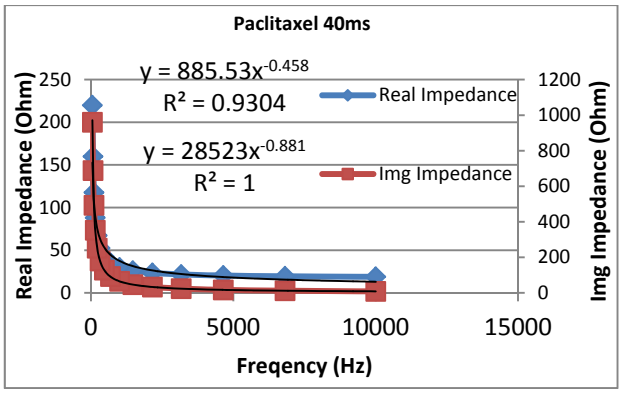


Figure 11a. Impedance Spectrum of electroporated sample – 200 V/cm, 40 ms pulses using Paclitaxel.

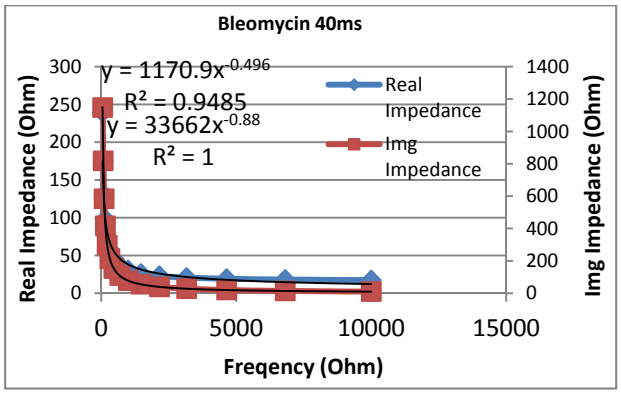
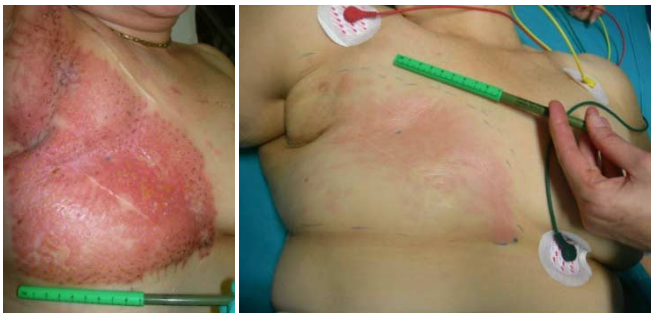


Figure 11b. Impedance Spectra of electroporated samples – 200 V/cm, 40 ms pulses using Paclitaxel (left) and Bleomycin.



(a) before treatment (b) 7 days after treatment

Figure 12. ECT Treatment of chest wall carcinoma.

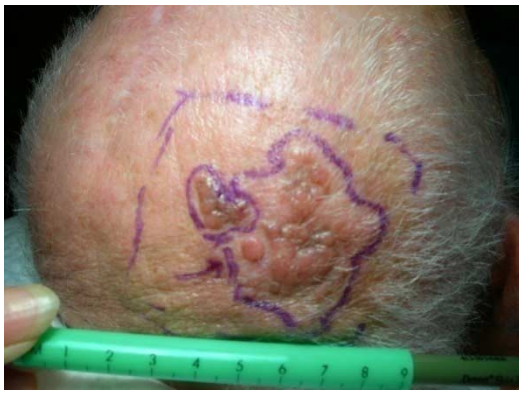


Figure 13. ECT treatment of head and Neck cancer (85 year old male patient).

CONCLUSIONS

- MCF-7, human adenocarcinoma breast cancer cells were electroporated with and without FDA approved chemotherapeutic drugs, Bleomycin and Taxol using low and high voltage pulses, 200 V/cm, 40 ms and 1200 V/cm, 200 μs pulses, for the first time.
- These cells were also studied using Tamoxifen, the classical hormone drug, for the first time.
- The viability results indicate the efficacy of the combination therapy, using drug and electrical pulses in controlling the proliferation of the cells.
- The FACS spectra indicate the death by apoptosis, the desired phenomena in cell death without causing swelling and other detrimental effects.
- The Western blot results indicate protein higher than the control for electroporated samples indicating the effectiveness of the technique.
- The impedance spectroscopy results compared with un-electroporated samples, such as control, pulse only, and drug only, indicate the enhanced conduction of the electroporated samples.
- There is a four to five orders of magnitude enhanced conduction between the electroporated and the un-electroporated samples indicating the efficacy of the technique in opening up pores allowing the transport of xenomolecules of chemo drugs, across the cell plasma membrane, which otherwise are impermeable to the drugs.
- The samples exhibited alpha and beta dispersion, indicating the typical dielectric dispersive nature of the biodielectric electrets, such as MCF-7 cells.
- The human clinical trials showed complete recovery in majority of the cases illustrating the excellent efficacy of this treatment modality.

In summary, our findings indicate that pulses as low as 200 V/cm of millisecond duration could be used to trigger cell death in the MCF-7 cancer cells, compared to the conventional 1200 V/cm, 100 μs pulses. Considering that very low doses of chemo drugs (50 millimolar in the case of Bleo and 9 nanomolar in the case of Taxol) were used, these results are encouraging for the transfer of this technique to clinics so patients will have another treatment modality with lower cost and fewer side effects, as indicated by the clinical trials.

ACKNOWLEDGMENT

We are very grateful to Mary Zheng for conducting part of this study (Figure 5) and Ryan Muir for training with Impedance spectroscopy.

REFERENCES

[1] A. M. Gonzalez-Augulo, F. Morales-Vasquez, and G. N. Hortobagyi, "Overview of resistance to systemic therapy in patients with breast cancer", Ch 1, pp. 1-22, in *Breast Cancer Chemosensitivity*, D. Yu and M-C Hung (Eds), Springer Sci Business Media, New York, USA, 2007.

[2] K. Kingsbury, "The changing face of breast cancer", Time Magazine, Oct 15, 2007.

[3] J. Ferlay, H. R. Shin, F. Bray, D. Forman, C. Mathers and D. M. Parkin, "Estimates of worldwide burden of cancer 2008: GLOBOCAN 2008", Int'l. J. of Cancer, Vol. 127, pp. 2893-2917, 2010.

[4] R. Heller, M. J. Jaroszeski, L. F. Glass, J. L. Messina, D. P. Rapaport, R. C. DeConti, N. A. Fenske, R. A. Gilbert, L. M. Mir, and D. S. Reintgen, "Phase I/II trial for the treatment of cutaneous and subcutaneous tumors using electrochemotherapy", Cancer, Vol. 77, pp. 964-971, 1996.

[5] J. Gehl and P. F. Geertsen, "Efficient palliation of haemorrhaging malignant melanoma skin metastases by electrochemotherapy", Melanoma Research, Vol. 10, pp. 1-5, 2000.

[6] J. O. Larkin, C. G. Collins, S. Aarons, M. Tangney, M. Whelan, S. O'Reiley, O. Breathenach, D. M. Soden, and G. C. O'Sullivan, "Electrochemotherapy: aspects of practical development and early clinical experience", Annals of Surgery, Vol. 245, No. 3, pp. 469-479, 2007.

[7] L. G. Campana, S. Mocellin, M. Basso, O. Puccetti, G. L. De Salvo, V. Chiarion-Sileni, A. Vecchiato, L. Corti, C. R. Rossi, and D. Nitti, "Bleomycin-based electrochemotherapy: clinical outcome from a single Institution's experience with 52 patients", Annals of Surgical Oncology, Vol. 16, pp.191-99, 2009.

[8] J. C. Weaver and K. H. Schoenbach, "Biodielectrics", IEEE Trans. Dielectr. Electr. Insul., Vol. 10, pp. 715-716, 2003.

[9] J. C. Weaver, "Electroporation of biological membranes from multicellular to nano scales", IEEE Trans. Dielectr. Electr. Insul., Vol. 10, pp. 754-768, 2003.

[10] T. J. Lewis, "A model for bilayer membrane electroporation based on resultant electromechanical stress", IEEE Trans. Dielectr. Electr. Insul., Vol. 10, pp. 769-777, 2003.

[11] H. Leontiadou, A. E. Mark, and S. J. Marrink, "Molecular dynamics simulations of hydrophilic pores in lipid bilayers," Biophysical J., Vol. 86, pp. 2156-2164, 2004.

[12] H. P. Schwan, "Dielectric properties of cells and tissues", in *Interactions between Electromagnetic Fields and Cells*, Eds. A. Chiabrera, C. Nicolin and H.P. Schwan, Plenum press, New York, 1985.

[13] K. Foster and H. Schwan, "Dielectric properties of tissues and biological materials: A critical review", Critical Rev. Biomedical Engineering, Vol. 17, pp. 25-104, 1989.

[14] M. P. Rols, M. Golzio, C. Delteil, and J. Teissie, "In vitro delivery of drugs and other molecules to cells", in *Electrochemotherapy, Electrogenetherapy, and Transdermal Drug Delivery*, M.J. Jaroszeski, R. Heller, and R. Gilbert (Eds.), Humana Press, Totowa, New Jersey, Ch. 4, pp. 83-97, 1999.

[15] S. Raffy and J. Teissie, "Surface charge control of electropereabilization and glycophorin electroinsertion with 1,2-diacyl-sn-glycero-3-phosphocholine (lecithin) liposomes", Eur. J. Biochem. Vol. 250, pp. 315-319, 1997.

[16] S. Li, X. Zhang, and X. Xia, "Regression of tumor growth and induction of long-term antitumor memory by interlukin 12 electrogenetherapy", J. Natl Cancer Inst., Vol. 94, pp. 762-768, 2002.

[17] S. M. Love, *Dr. Susan Love's Breast Book*, Capo Press, 3rd ed., 2000.

[18] Y. Yu and Q. Li, "Electrochemical studies of paclitaxel interaction with tubulin", Chinese Chemical Letters, Vol. 11, pp. 351-352, 2000.

[19] D. S. Alberts, H.-S. G. Chen, M. Mayersohn, D. Perrier, T. E. Moon, and J. F.Gross, "Bleomycin pharmacokinetics in man", Cancer Chemotherapy and Pharmacology, Vol. 2, pp. 127-132, 1979.

[20] K. W. Branner and R. W. Sonntag, "Bleomycin in testicular teratomas", J. Cancer Research and Clinical Oncology, Vol. 84, pp. 0171-5216, 1975.

[21] M. Baum, J. A. Dossett, J. S. Patterson, F. G. Smiddy, A. Wilson, D. Richards, D. M. Brinkley, K. Mcpherson, R. D. Rubens, B. A. Stoll, J. C. Lea, and S. H. Ellis, "Improved survival among patients treated with adjuvant tamoxifen after mastectomy for early breast cancer", Lancet, 2(8347):450, 1983.

[22] Y. Shang, X. Hu, J. DiRenz, M. A. Lazar, and M. Brown, "Cofactor dynamics and sufficiency in estrogen receptor-regulated transcription", Cell, Vol. 103, pp. 843-852, 2000.

[23] S. Massarweh, C. K. Osborne, C. J. Creighton, L. Qin, A. Tsimelzon, S. Huang, H. Weiss, M. Rimawai, and R. Schiff, "Tamoxifen resistance in breast tumors is driven by growth factor receptor signaling with repression of classic estrogen receptor genomic function", Cancer Res., Vol. 68, pp. 826-833, 2008.

[24] A. Hurtado, K. A. Holmes, T. R.. Geistlinger, I. R. Hutcheson, R. I. Nicholson, M. Brown, J. Jiang, W. J. Howat, S. Ali, and J. S. Carroll, "Regulation of ERBB2 by oestrogen receptor-PAX2 determines response to tamoxifen", Nature, 456(7222):663-666, 2008.

[25] C. K. Osborne, V. Bardou, T. A. Hopp, G. C. Chamness, S. G. Hilsenbeck, S. A. Fugua, J. Wong, D. C. All red, G. M. Clark, and R. Schiff, "Role of the estrogen receptor coactivator AIB1 (SRC-3) and HER-2/neu in tamoxifen resistance in breast cancer", J. Natl. Cancer Inst., Vol. 95, pp. 353-361, 2003.

[26] B. L. Ibey, A. G. Pakhomov, B. W. Gregory, V. A. khorokhorina, C. C. Roth, M. A. Rassokhin, J. A. Bernhard, G. J. Wilmink and O. N. Pakhomova, "Selective cytotoxicity of intense nanosecond-duration electric pulses in mammalian cell", Biochim. Biophys. Acta, 1800(11), p. 1210-1219, 2010.

[27] http://en.wikipedia.org/wiki/western_blot, 2011.

[28] T-S. Huang, C-H Shu, Y. Chao, S-N Chen, and L-L Chen, "Activation of MAD 2 ceheckprotein and persistence of cyclin B1/CDC 2 activity associate with paclitaxel-induced apoptosis in human masopharyngeal carcinoma cells", Apoptosis, Vol. 5, pp. 235-241, 2005.

[29] R. Y. Wang, T. Zhang, Q. Bao and D. M. Rawson, "Study on fish embryo responses to the treatment of cryoprotective chemicals using impedance spectroscopy", Eur. Biophys. J., Vol. 35, pp. 224-230, 2006.

[30] A. Soley, M. Lecina, X. Gamez, J. J. Cairo, P. Riu, X. Rosell, R. Bragos and F. Godia, "On-line monitoring of yeast cell growth by impedance spectroscopy", J. Biotechnol., Vol. 118, pp. 398-405, 2005.

[31] K. S. Osterman, "Non-invasive assessment of radiation injury with electrical impedance spectroscopy", Physics in Medicine and Biology, Vol. 49, pp. 665-683, 2004.

[32] H. P. Schwan, "Electrical properties of tissues and cell suspensions", Adv. Biol. Med. Phys., Vol. 5, pp. 147-209, 1957.

[33] R. Pethig and D. B. Kell, "The passive electrical properties of tissues and cell suspensions", Phys. Med. Biol., Vol. 3, pp. 933-70, 1987.

[34] L. M. Mir, J. Gehl, G. Sarsa, C. G. Collins, J-R Garbay, V. Billard, P. F. Geertsen, Z. Rudolf, G. C. O'Sullivan, and M. Marty, "Standard operating procedures of the electrochemotherapy: Instructions for the use of bleomycin or cisplatin administered either systematically or locally and electric pulses delivered by the CliniporatorTM by means of invasive or noninvasive electrodes", EJC Supplements, Vol. 4, pp. 14-25, 2006.

[35] C. Holzapfel, J. Vienken, and U. Zimmermann, "Rotation of cells in an alternating electric field: Theory and experimental proof", J. Membrane Biology, Vol. 67, no. 1, pp. 13-26, 1982.

[36] S. S. Bacus, A. V. Gudkov, M. Lowe, L. Lyass, Y. Yung, A. P. Komarov, K. Keyomarsi, Y. Yarden, and R. Seger, "Taxol-induced apoptosis depends on MAP kinase pathways (EPK and p38) and is independent of p53", Oncogene, Vol. 20, No. 2, pp. 147-155, 2001.

[37] D. A. Dean, T. Ramanathan, D. Machado, and R. Sundararajan, "Electrical Impedance Spectroscopy study of biological tissues", J. Electrostatics, Vol. 66, p. 2008, 165-177 2008.

[38] A. Soley, M. Lecina, X. Gamez, J. Cairoa, P. Riu, X. Rosell, R. Bragos, and F. Godia, "On-line monitoring of yeast cell growth by impedance spectroscopy", J. Biotechnology, Vol. 118, pp. 398-405, 2005.

[39] B. Sayers, *Impedance Spectroscopy Theory, Experiment, and Applications*, Commercially Available Impedance Measurement Systems, J. Wiley and Sons, Inc., 2nd ed., pp. 168-192, 2005.

Raji Sundararajan earned the B.E. (Honors) degree with gold medal and the University of Madras endowment prize in EEE from the Government College of Engineering, Salem, the M.E. degree in high voltage engineering from IISc, Bangalore, India and the Ph.D. degree in EE from Arizona State University, Tempe, AZ, USA. She is a Professor at the Electrical and Computer Engineering Technology (ECET) Dept., Purdue University. She served at Arizona State University, USA and The Dept. of Space, India. She has published over 130 papers in journals, international conferences, book chapters, IEEE Standards, and a book. She has chaired the IEEE CEIDP in 2010 and 2011 and was president of the Electrostatics Society of America in 2009-2011.

Funian Xiao completed the M.S. degree in the ECET Dept., Purdue University. His thesis work was on the pioneering work of electroporation of MCF-7, human breast carcinoma cells using FDA approved breast cancer drugs including Paclitaxel, Tamoxifen, and Bleomycin. He is currently serving at GE Medical Systems, USA.

Therese S. Salameh is a graduate student in the Department of Biological Sciences at Purdue University. She received the B.A. degree from Clark University in Worcester, MA and the M.A. degree from Purdue University, West Lafayette, IN., USA. Her current research focuses on the underlying biological mechanisms involved in the relationship between obesity and breast cancer. She examines changes in tissue fatty acid composition in relation to various stages of tumor growth and development to identify links between diet and breast cancer.

Lisa M. Reece is the Biosafety Officer and Lab Director for the Indiana Clinical Translational Sciences Institute at the BioNanotechnology Core Facility in the Birck Nanotechnology Center at Purdue University. She is also a member of the Oncological Sciences Center at Purdue. Lisa is currently pursuing her PhD in the Dept. of Basic Medical Sciences. She obtained the B.S. degree in microbiology from the University of Texas at El Paso. Ms. Reece has 4 years of industrial microbiology experience with Baxter Scientific, Inc., Baltimore Spice Co., and Promega, Inc. She has over 20 years of academic experience in immunology, oncology, and infectious disease research with UT EL Paso, William Beaumont Army Medical Center, UT MD Anderson Cancer Center, and UT Medical Branch. Her research background is multidisciplinary including high speed flow cytometry and single cell sorting and advanced single cell analysis.

Ignacio G. Camarillo is an Associate Professor in the Department of Biological Sciences at Purdue University. He received the Ph.D. degree from Wayne State University, Detroit, USA. He was a National Cancer Institute Postdoctoral Fellow at the University of California Santa Cruz, USA. His current research focuses on the underlying biological mechanisms involved in the relationship between obesity and breast cancer. Dr. Camarillo is a member of the Purdue Center for Cancer Research. He is director for Purdue undergraduate research programs LSAMP and the Cancer Center SROP.

James F. Leary is Professor of Nanomedicine and a professor in the Department of Basic Medical Sciences and the Weldon School of Biomedical Engineering at Purdue University. He is also a Member of the Center for Cancer Research, the Bindley Biosciences Center, and the Birck Nanotechnology Center at Discovery Park. He holds 9 U.S. Patents, with 4 currently pending and is the author or co-author of 135 papers and book chapters in the fields of high-throughput technologies, minimal residual disease monitoring, developmental immunology, cancer research, and nanomedicine. He is a Fellow of the American Institute for Medical and Biological Engineering. He was awarded the International Journal Nanomedicine Distinguished Scientist Award. He is a Fellow of International Society for Optical Engineering for achievements in high-speed flow cytometry and nanomedicine.

Kevin J. Otto received the B.S. degree in chemical engineering from Colorado State University in 1997, the M.S. degree in bioengineering in 2002 and the Ph.D. degree in bioengineering in 2003 from Arizona State University, Tempe. From 2003 to 2006 he was a post-doctoral Fellow in the Department of Biomedical Engineering and the Department of Otolaryngology at the University of Michigan, Ann Arbor where his work focused on brain-machine interfaces and cochlear implants. He is currently an Associate Professor in the Department of Biological Sciences and the Weldon School of Biomedical Engineering at Purdue University. His research interests include neuroprostheses, systems neuroscience, and neurotechnologies.

Luca G. Campana is a surgical oncologist at the Sarcoma and Melanoma Unit of the Veneto Region Oncology Research Institute (IOV-IRCCS) of Padova, Italy. He earned his medical degree from the University of Padova. His clinical interests lie around the prevention and therapy of malignant melanoma, soft tissue sarcoma and breast cancer. He is now pursuing a Ph.D. at the University of Padova and his research interest is focused on locoregional chemotherapies and in particular on the clinical applications of electrochemotherapy in cancer patients.