Impedance Spectroscopy Studies of Electroporated Human Breast Cancer Cells

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***Abstract*—The passive, electrical impedance characteristics of electroporated, MCF-7 human breast cancer is described in this paper. For this purpose, an Autolab impedance spectroscope was used. The electroporation protocol consisted of 200V/cm, 40ms, and 1200V/cm, 200μs pulses. The impedance characteristics of these cells displayed a negative fractal power law as expected for dielectrics. They also exhibited single dispersion Cole-Cole model. Our experiments demonstrated that the various electroporation treatment parameters result in the different curvatures of Cole-Cole model. This phenomenon proves that the effects of electroporation drug delivery to cells could be controlled in real time by monitoring impedance characteristics.**

I. Introduction

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) [1]. These changes are due to the permittivity and conductivity (or resistivity) contributions of various relaxation processes that follow due to the variation in the external applied field.

In this paper, we present the electrical properties of biological cells over the investigated frequency range of 50 to 10,000Hz using impedance spectroscopy (IS), which 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 need be 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 [2, 3]. IS measures the electrical properties of any material, i.e. the conductance (or resistance) and the reactance as a function of applied alternative current (ac) frequency.

II. Materials & Methods

A. 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 90% RPMI 1640 media with 10% fetal bovine serum (ATCC) and 1% penicillin/streptomycin (Invitrogen) and incubated in a 5% CO_2 atmosphere at 37°C.

For electroporation, cells were washed twice with 1x PBS 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.

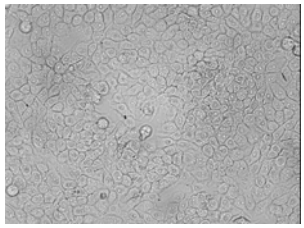

Figure 1. MCF-7 Human ER+ Breast Adenocarcinoma cell line.

B. Chemodrugs

a) Bleomycin:

Bleomycin (Bleo) is a FDA approved chemo drug 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. Common side effects include fever, chills, skin reactions, hair loss, alopecia, and gastrointestinal issues. In this research, Bleomycin is used to treat human breast cancer cells, MCF-7 with a low dose of 50μM concentration, because electrochemotherapy requires less dose of drug than standard clinical chemo therapy doses. In addition, due to the reduced dose, there is lower degree of toxicity and side effects.

b) Paclitaxel

Paclitaxel (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. 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, so less dose (9nM) could be used and hence reduce side effects.

C. Electroporation Technique

The use of electroporation for the delivery of Bleomycin is a method, which is approved for veterinary use in the European Union. Moreover, clinical trials are underway for the delivery of drugs into the skin [7]. In our previous work, we

successfully delivered the hormone drug Tamoxifen using electroporation with 200 V/cm, 10-40ms, 8 pulses at one second intervals [8]. The same intensity was used in our initial effort in this study. In addition we also used the 1200V/cm, 200µs pulses [7, 9]. BTX ECM 830 (Genetronics, Inc, San Diego, CA), square wave electroporator with 0.4cm electrode gap cuvettes were used for this purpose.

D. Impedance Spectroscopy

AC impedance at room temperature was measured using a PGSTAT100 (Autolab) high voltage potentiostat/ galvanostat with a compliance voltage of 100 V and a maximum current of 250 mA. It gives 40 data points for a frequency range from 0.5Hz to 100kHz. A sine voltage of 25mV is applied and the data were collected from 46Hz to 10kHz. The samples were held in a 4mm electrode gap cuvette and the two leads were connected to the cuvette. The samples were scanned at 15 frequency points over the frequency range of 46Hz to 10,000Hz. Electrical impedance was displayed as Real Z (R, the resistive component in Ohm) and Img Z (the capacitive reactance component in negative Ohm).

III. Results & Discussion

A. Impedance Plots

Figure 2 presents the display of the frequency dependent dielectric constant and conductivity of control (no treatment) sample of the MCF-7 human adenocarcinoma breast cancer cells over 50Hz to 10,000Hz. The capacitive reactance (Xc), the imaginary component, represents dielectric constant which characterizes the ability of the cell to store electricity, 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. These two properties solely characterize the electrical characteristics of matter [1]. The magnetic properties of the cells are not considered as they are virtually identical with those of free space.

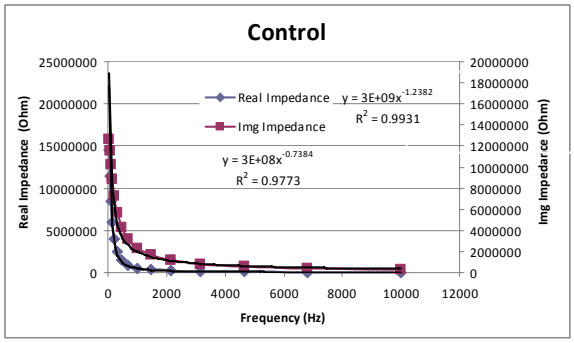

Figure 2. Impedance spectrum of MCF-7 Breast cancer cells – Control (no treatment). Negative Img Z is shown.

The curves display two unique features: a) the dielectric constant reaches enormous values of more than a million as the frequency decreases below 100Hz, b) the data change in two distinct steps at the measured frequency range, each of them of a clearly "dispersive" (frequency dependent) nature. These are known as alpha (α) dispersion in the frequency range up to 1000Hz and beta (β) dispersion in the frequency range >1,000Hz [1]. 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. 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.

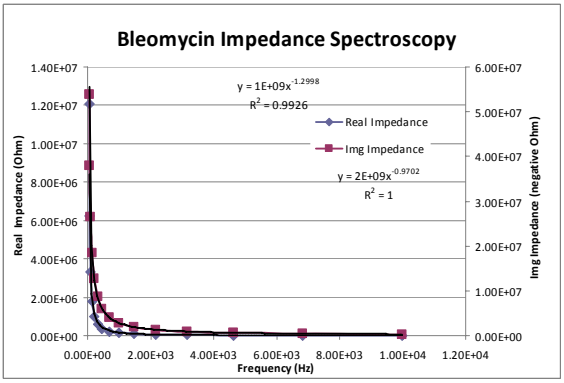

Figure 3. Impedance spectrum of MCF-7 Breast cancer cells – Control (no treatment). Negative Img Z is shown (due to capacitive reactance).

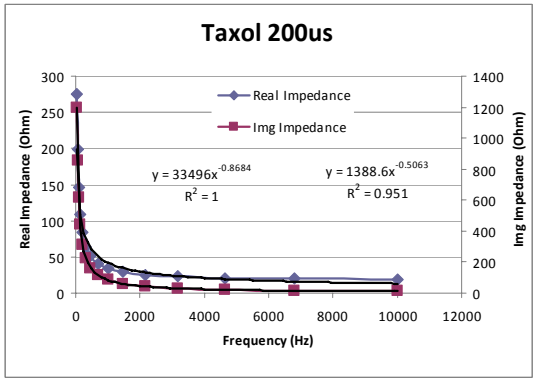

Figure 4. Impedance spectrum of MCF-7 Breast cancer cells – Electroporated at 1200V/cm, 200us, 8 pulses at one second interval illustrating the enhanced conductance and hence the reduction in R and xc compared to control and drug only samples in Figure 3. There is a 4order of magnitude difference between the electroporated and un-electroporated samples indicating the enhanced permeation of poration of the cell membrane at the applied pulse conditions. Negative Img Z is shown.

Figure 3 shows the impedance characteristics of the drug only samples. They closely follow the control sample, both in magnitude and trend. Figure 4 shows that of the electroprated sample using 1200V/cm, 200us pulses (eight pulses at one second interval). It can be seen the dramatic decrease in the magnitude of the impedance, both the real and the imaginary components indicating the enhanced increase in conduction or permeability of the cell membrane due to the application of

pulses. The magnitudes were in the order of seven digits for control and drug only samples, while they are in lower three digits for the electroporated samples with Taxol chemodrug. Similar results were obtained for electroporated samples with and without drug (data not shown).

It can be seen that all these impedance plots follow α and β dispersions as well as negative (inverse) fractal power law typical of dielectrics [1, 10, 11]. The variations in indices reflect the differences between electroporated and the control and drug only samples. The electroporated samples have a lower index of around negative 0.5, while the control and drug only samples have indices >0.5. These results correlate very well with the previous results reported by Schwan [10].

B. Cole-Cole Impedance Plots

Figures 5-7 show the Real Z vs Img Z complex plane locus, known as Cole-Cole impedance plots [10, 12] for the un-electroporated and electroporated samples. Here, the imaginary part of the complex impedance of the cells is plotted against the real part, for each frequency. The low frequency data are on the right side of the plot, having higher magnitudes than the high frequency data which are on the left side, as the impedance falls with increase in frequency as seen in Figures 2-4.

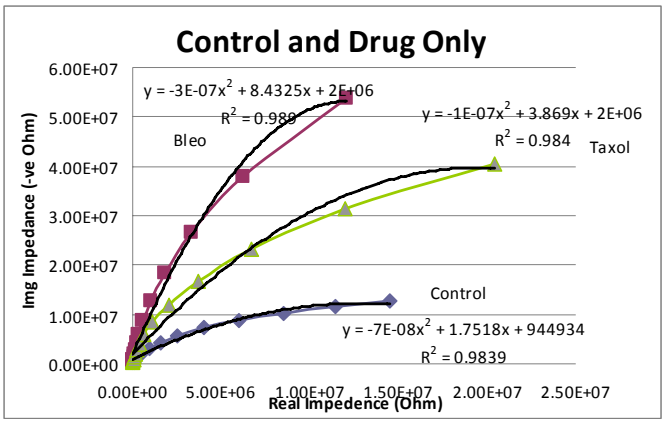

Figure 5. Cole-Cole (Resistance vs Reactance) Plot indicating the high magnitudes of R and Xc for control and drug only.

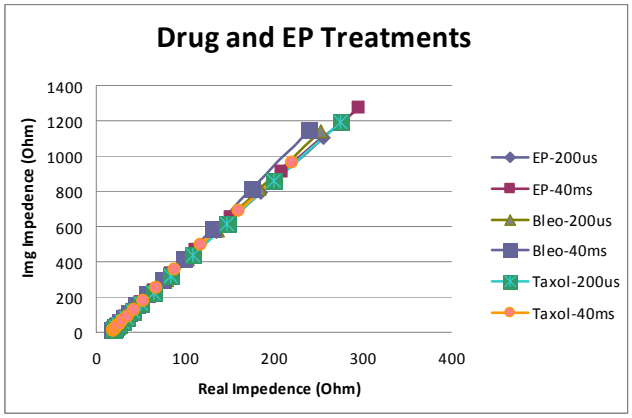

Figure 6. Comparison of effects of low intensity, long duration pulses vs high intensity, short duration pulses.

For truly complex impedance, the Cole-Cole plot is a half circle (due to second order variation) [10, 12]. In our research, the un-electroporated samples approach a semicircle (Figure 5). The electroporated samples, with and without drug, are only approaching an arc (Figure 6). A fitting of trend line (Figure 7) showed the trend of a semicircle with R^2 =1. These results again clearly illustrate the changes in the cell electrical properties due to the electroporation pulses.

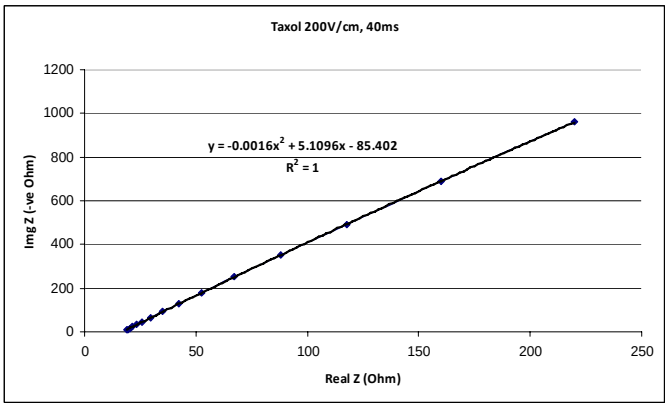

Figure 7. Cole-Cole plot for Taxol Chemodrug, electroprated using eight, 200V/cm, 40ms pulses at one second interval. There is a better fit using 2nd order polynomial than linear straight line indicating the approach to a semicircle, similar to the un-electroporated samples in Figure 5.

C. Bode Plots

Total impedance, Z vs. frequency and phase angle, ϕ vs. frequency are known as Bode plots. In these plots, magnitude of Z decreases with increase in frequency and approach R at high frequencies [13, 14]. At lower frequencies the phase, ϕ is equal to 90° as for a pure capacitor, but at higher frequencies it decreases approaching the phase angle of resistance or that combination of R and C. The Bode plots obtained in our measurements are illustrated in Figures 8a and b and 9a and b. Figure 8 shows the Bode plots for the samples with drug, bleo only.

Figure 9 shows the bode plots for the electroporated sample with chemo drug bleo, using eight 200V/cm, 40ms pulses. While the Z vs. frequency plots follow the typical style in both cases, the phase angle vs. frequency of the drug only sample has a trend opposite to that mentioned above, i.e., there is an increase in the phase angle with frequency, while the electroporated sample with the drug follows the expected trend, namely, decrease in phase angle with increase in frequency from 78° to 30°. The control sample (with no treatment) also exhibited similar trend as the drug only while the other electroporated sample with eight 1200V/cm, 200us pulses exhibited the trend similar to Figure 9 (data not shown). This needs further analysis.

IV. Conclusions

- MCF-7, human adenocarcinoma breast cancer cells were electroporated with and without chemotherapeutic drugs, Bleomycin and Taxol using 200V/cm, 40ms and 1200V/cm, 200μs pulses.
- The impedance results compared with un-electroporated samples-control (no treatment-no pulse and no drug) and drug only, indicate the enhanced conduction of the electroporated samples.

- These results correlate very well with those obtained for tissues [14].

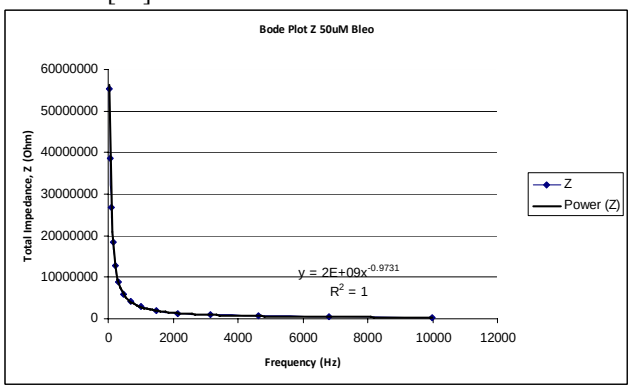

Figure 8a. Total Z vs frequency Bode Plot for drug only sample.

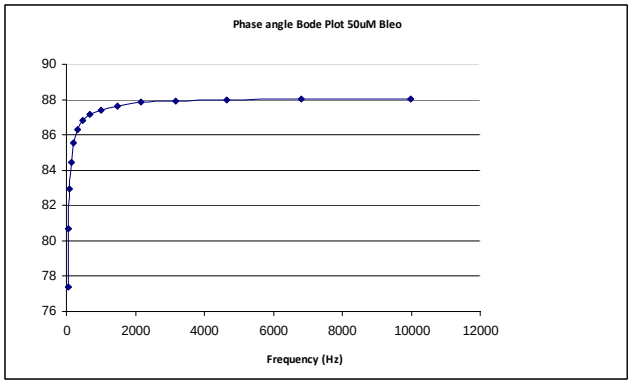

Figure 8b. Phase angle vs. frequency Bode Plot for drug only sample.

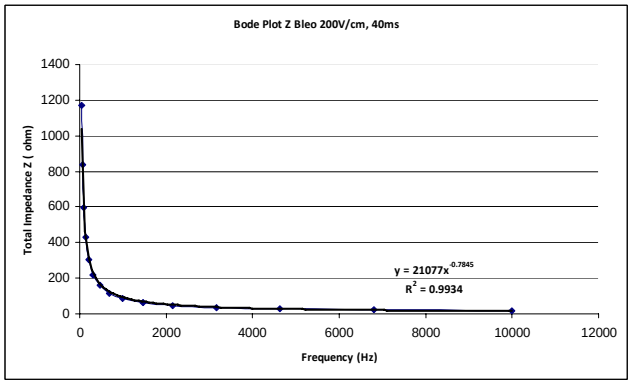

Figure 9a. Total Z vs. frequency Bode Plot for electroporated drug sample.

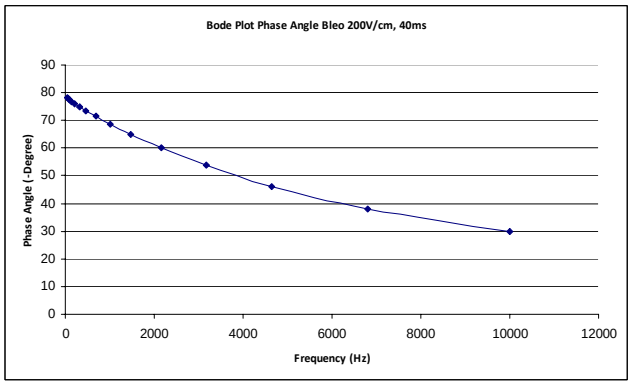

Figure 9b. Phase angle vs. frequency Bode Plot for electroporated drug sample.

- There is a 4 to 5 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 true dielectric dispersive nature of the MCF-7 cells.
- The Cole-Cole plots approached semicircle, indicating the true dielectric nature of these cells.

Our findings indicate that pulses as low as 200 V/cm but with millisecond duration could be used to trigger cell death in the MCF-7 cancer cells, compared to the conventional 1200V/cm, 100μs pulses. Considering that very low doses of chemo drugs (50millimolar in the case of Bleo and 9nanomolar in the case of Taxol) were used, these results are encouraging to apply this technique so patients will have fewer side effects.

By studying the electrical impedance of various cells over a frequency range, its frequency-dependent dielectric behavior could be determined and used for various applications including pathology, diagnostics, and treatment using electrical pulses.

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