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The Dielectric Properties of Electroporated Human Breast Cancer Cells

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Abstract: The dielectric properties of biological cells and tissues are of interest for developing therapeutic medical applications, especially when they are exposed to electrical fields as in the case of electroporation, a technique where-in high intensity, short duration pulses are applied to enhance the uptake of normally impermeable molecules, such as hydrophilic chemo drugs. Electrical impedance spectroscopy is a powerful tool to study these as the impedance signatures of biological cells is characterized by the frequency-dependent changes (dielectric dispersion), due to their frequency dependent permittivity and conductivity (resistivity). This paper presents the impedance spectroscopy studies of electroporated human breast carcinoma cells using FDA approved chemo and hormone drugs. For this purpose, an Autolab high voltage potentiostat was used. A sine voltage of 25mV is applied and the data were collected for a range of frequencies. The dielectric response of breast cancer cells indicate that they have alpha and beta dispersions. The fractal index value and other results obtained in this research compare well with previous results. The results indicate both quantitatively and qualitatively the enhanced conduction of the electroporated cells corroborating the uptake of chemo drug molecules. This indicates that impedance spectroscopy could be used for characterizing cells and tissues for treatment using electrical pulses.

Key Words: Electroporation, MCF-7 human breast cancer cells, impedance spectroscopy, dielectric dispersion.

INTRODUCTION

Impedance Spectroscopy is a versatile technique used for characterization of electrical properties of materials in almost all fields including biology, chemical, and material sciences [1-3]. It can measure many variables from dielectric properties to microstructure in biological cells. The cells respond the electrical signals applied by the impedance spectroscope to stimulate the cells. The response consists of three different stages. First, the ions in the media around cells transport through the media based on the direction of electric field. Second, the ions in the media transfer from the cell membrane interfaces to charged or discharged particles in the cytoplasm. Third, the charged particles, ions or drug particles, flow through the cytoplasm and media. The current of the charged particles depends on the impedance and conductance of the media, the cell membrane, the cytoplasm, and the reaction rates among these material interfaces [2-3].

A simpler electrical modeling of a biological cell consists of the parallel combination of resistance and capacitance, the resistance corresponding to the conductivity (or resistivity) and the capacitance corresponding to the relative permittivity of the cell [1]. The impedance of the electrical model of cell is governed by equation (1), as

$$Z_a = Y_a^{-1} = \frac{R}{RY_a} = \frac{R}{[1 + j\omega RC]} \qquad (1)$$

Thus they have both components, real Z and imaginary (Img) Z, which is negative due to the capacitance. Since the electrical conductivity and the relative permittivity of a cancer cell depends on both the physicochemical bulk properties, such as amount of fluids and ions in it, and its micro structural properties, they will vary with respect to frequency [4]. This enables the investigation of the impedance of biological cells at various frequencies using impedance spectroscopy and characterize their frequency-dependent changes (dielectric dispersion) [5-8]. They normally undergo the following three dispersions related to the various frequency (f) domains of relaxations [8]: alpha (α) dispersion at f < 1kHz, beta (β) dispersion at 1kHz < f < 100MHz) and gamma dispersion at f > 1GHz. The α dispersion is attributed to the ions; the β dispersion due to the orientation of diploes and capacitive responses of the plasma cell membrane.

In this research, we studied the impedance signatures of the human breast cancer cells with and without electroporation and anticancer drugs, the application of high intensity, short duration electrical pulses to enhance the uptake of normally impermeable or low permeable hydrophilic chemo drug molecules for treating those tumors which are inoperable, recurrent and chemo-resistant. This will help quantify the enhanced permeability of drug transport across the plasma membranes.

MATERIALS & METHODS

The cells

MCF-7 is a cell line, isolated from a 69 year old, Caucasian woman. These cells are widely used for in vitro breast cancer studies as these cells have

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several ideal characteristics particular to the mammary epithelium, including retaining their estrogen sensitivity [9]. They form a highly aggressive cell line [10]. These cells were prepared for electroporation as in [11] using RPMI medium ATTC, Manassas, VA).

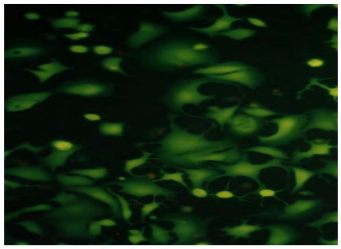

Fig. 1. Micrograph of MCF-7 breast cancer cells [9].

The anticancer drugs.

FDA approved, commercial chemo drug Paclitaxel (Taxol) and the classical hormone drug, Tamoxifen, for breast cancer 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 [12, 13], including fever, chills, skin reactions, hair loss, nausea, vomiting, diarrhea, and muscle pain, alopecia, gastrointestinal issues, etc., thus, ideal candidates for EP.

Taxol is a chemotherapeutic drug that is currently used worldwide to treat ovarian, lung and breast cancer [14]. It belongs to a chemical group, called taxanes, whose mechanism of action depends on their high affinity binding to microtubules and causing them from proliferation (inhibits cell growth) [15]. Taxol compounds are small organic molecules that change in their electronic structure, orientation, and isomerization of their tails, and are characterized as rather large diploes. These modifications enhance their pharmacological activity of this very potent drug [16]. Fig. 2a shows its chemical/atomic structure [17].

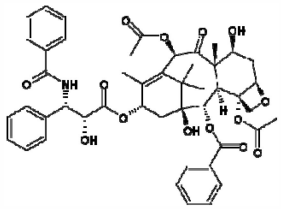

Fig. 2. Chemical/atomic structure of Paclitaxel [16].

Tamoxifen is the Cadillac of breast cancer hormone drugs [12, 13]. Its chemical/atomic structure is shown in Fig. 2b. 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 and reduces the cancer cell proliferation [14].

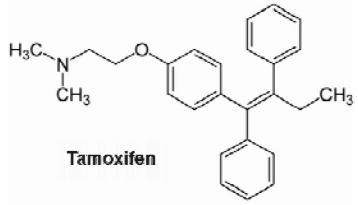

Fig. 2b. Chemical/Atomic Structure of Tamoxifen.

The Electroporation Technique

For efficient electroporation, there has to be sufficient amount of chemotherapeutic drug present when the pulses are applied and the electric pulses have to be of appropriate magnitude to create transient (reversible) 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. The electric field intensity, E and the duration of the pulse, T, constitute a relationship, ET=k, a constant [18]. This means if E is smaller, T has to be longer and vice versa. In this project, both low intensity, long duration (200V/cm, 40ms) and high intensity, short duration (1200V/cm, 200μs) pulses were tested. Table 1 shows the various conditions studied. These conditions were chosen because these pulses were successfully used by other researchers and us in both in vitro and in vivo studies including clinical trails [19-21]. Eight pulses at one second interval were applied using a BTX ECM 830 (Genetronics, Inc, San Diego, CA), square wave electroporator with 0.4cm electrode gap cuvettes.

Table 1 Samples and parameters of electroporation using FDA approved chemo drugs Paclitaxel and Bleomycin

Sample	Electric field intensity (V/cm)	Pulse duration	Pulse number	Pulse interval (s)
Control (no treatment)	0	0	0	0
Drug only	0	0	0	0
EP-40ms	200	40 ms	8	1
EP-200μs	1200	200 μs	8	1

Impedance Spectroscopy

A PGSTAT100 (Autolab) high voltage potentiostat/ galvanostat with a compliance voltage of 100 V and a maximum current of 250 mA was used at room temperature. 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 of the potentiostat were connected to the cuvette [11].

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 imaginary (Img) Z (the capacitive reactance component in negative Ohm). In the case of Tamoxifen hormone

drug, the frequency range used was 0.01 to 10,000Hz.

RESULTS & DISCUSSION

Fig. 3 shows the total impedance vs. frequency of the drug only sample, using 9nM Taxol chemodrug. It can be seen that the magnitude of Z is very high at lower frequencies and it reduces drastically with increasing frequency. This is due to the capacitive nature of the cell, Xc = 1/2πfC. With increase in f, Xc, reduces drastically and dominates over the resistance value, which also decreases as illustrated in Fig. 4, for Taxol only, showing the reduction of the individual components, Real Z and Img z, with frequency.

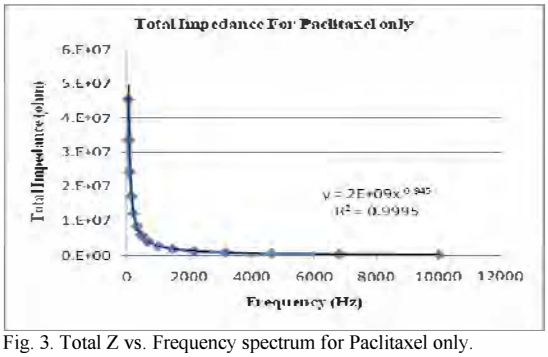

Fig. 3. Total Z vs. Frequency spectrum for Paclitaxel only.

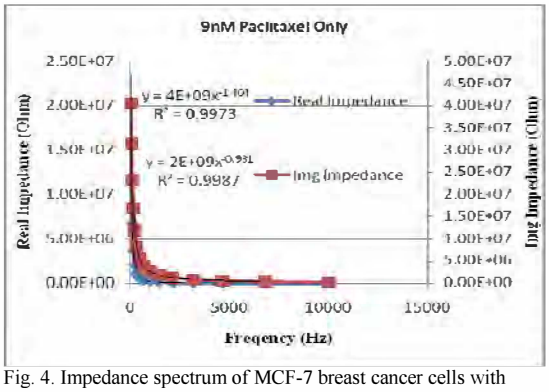

Fig. 4. Impedance spectrum of MCF-7 breast cancer cells with Taxol drug only.

Figs. 5 and 6 show the impedance spectra of the electroporated samples with the same dose of Taxol for the two conditions, 1200V/cm and 200V/cm. It can be seen that the magnitudes are reduced by orders of magnitude for the electroporated samples, illustrating the enhanced permeability enabling the uptake of the normally impermeable or low permeable drug across the plasma cell membrane. This correlates well with the up to 1000x increased efficacy seen in the electrochemotherapy efficacies for a given drug dose [19].

All these sample have the α dispersion around 1000Hz and the beta dispersion, above 1000Hz, typical of biodielectrics [8, 22].

Also, these samples fit the fractal, negative, power-law frequency dependency reported by the pioneering work of Schwan [8], where in a value of -0.3 to -0.5 was given for frequencies from below 1Hz to above 10kHz. Our samples show a value of -0.46 for the 200V/cm, 40ms sample, while it is -0.5 for the 1200V/cm sample, indicating the biodielectrica nature of these cells. The drug only samples show the power-law constant around 1, illustrating their perfect dielectric status, compared to the leaky dielectric nature of the electroporated samples. These results very well with those obtained for a rat lung tissue (Fig. 7 [23]).

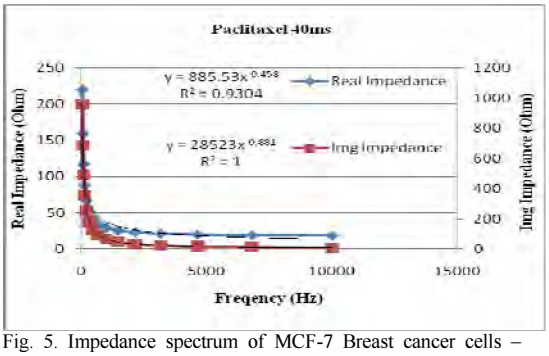

Fig. 5. Impedance spectrum of MCF-7 Breast cancer cells – Electroporated with 9nM Taxol at 200V/cm, 40ms, 8 pulses.

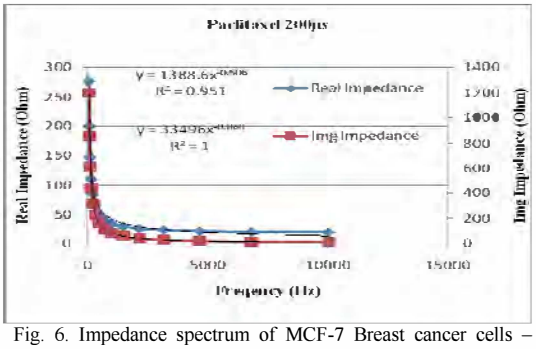

Fig. 6. Impedance spectrum of MCF-7 Breast cancer cells – Electroporated with 9nM Taxol at 200V/cm, 40ms, 8 pulses.

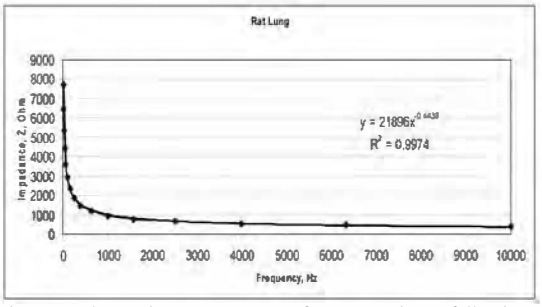

Fig. 7. Total Impedance Z spectrum of Rat Lung tissue, following the Power law, with a Constant of -0.44 (R2 = 0.9974) [23].

Fig. 8 shows a comparison of the real Zs of the drug only and the electroporated sample (1200V/cm) with the same dose of drug, illustrating the enhanced permeability.

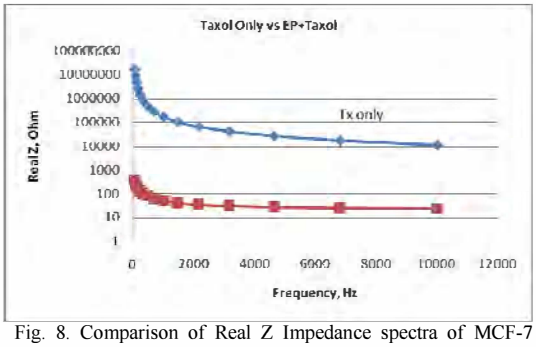

Fig. 8. Comparison of Real Z Impedance spectra of MCF-7 Breast cancer cells – Taxol only vs. Electroporated with Taxol at 1200V/cm, 200µs pulses. Drug dose is9nM in both cases.

Fig. 9 shows a comparison of the variation of tan delta (δ), the dielectric loss tangent with frequency for the drug only sample vs. the electroporated Taxol sample with 1200V/cm pulses at 9nM dose. The tan δ is calculated as the ratio of the real value to Img value, based on the equation [24],

$$\tan\delta = \frac{\sigma}{2\pi\varepsilon_0\varepsilon_r} = \frac{Ic}{Id} \qquad (2)$$

where Ic corresponds to the conduction current, and hence Real Z, and Id corresponds to the displacement current and hence, the Img Z. The trend varies for the drug only sample with very low dielectric loss with a small change with frequency. It increased with frequency for the electroporated sample, and the magnitudes are also relatively high compared to drug only samples, indicating higher losses due to the transient dielectric breakdown of the cells with the application of the high intensity, short duration pulses.

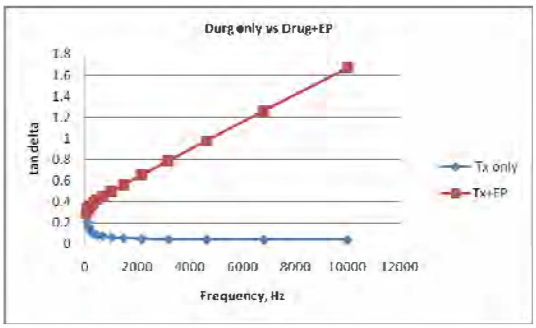

Fig. 9. Comparison of tan δ for the electroporated (1200V/cm, 200µs pulses) and drug only samples, at 9nM.

Fig. 10 shows the Cole-Cole plots [22, 25] for the Tamoxifen drug samples, the Real Z vs the Img Z. Fig. 10a shows drug only using 1µM Tamoxifan and Fig. 10b shows that of electroporated with 200V/cm, 40ms pulses. In this case also, there is reduction in the impedance values for the electroporated sample compared to that of the drug only sample and they also follow the second order arc of a circle that is representative of the loci of the dielectric behavior [22, 25]. Fig. 11 shows that of the Taxol sample, pulsed with 200V/cm, 40ms, pulses whose magnitudes are similar to those of Tamoxifen [11]. These results correlate with the published results of previous researchers [22, 25].

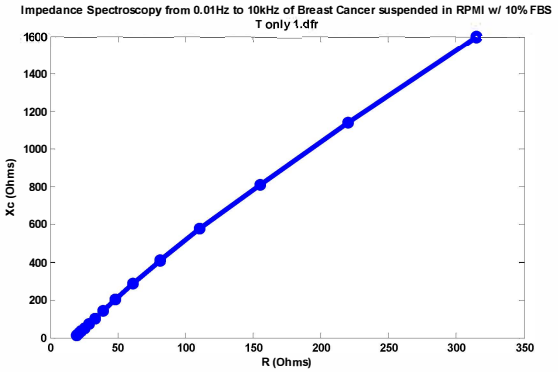

Fig. 10a. Cole-Cole Plot of Tamoxifen only for the frequency range 0.01 to 10000Hz. The high frequency values are to the left

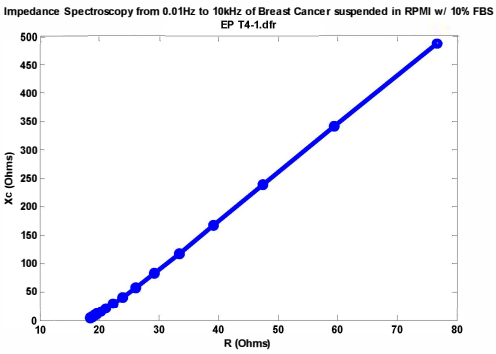

Fig. 10b. Cole-Cole Plot of Electroporated sample using 200V/cm, 40ms pulses with Tamoxifen ofor the frequency range 0.01 to 10000Hz. The high frequency values are to the left.

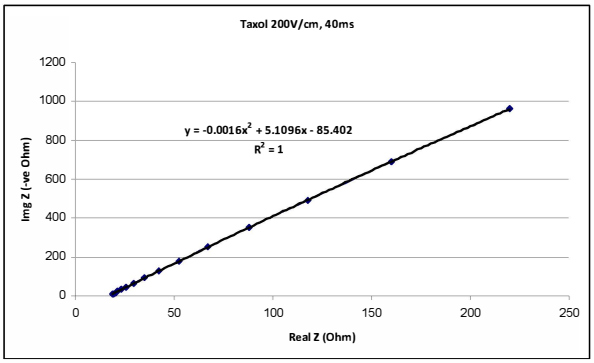

Fig. 11. Cole-Cole plot for Taxol Chemodrug, electroprated using eight, 200V/cm, 40ms pulses at one second interval [11].

CONCLUSIONS

- MCF-7, human adenocarcinoma breast cancer cells are respond to low voltage, long duration and high voltage short duration pulses for enhanced uptake of chemo and hormone drugs.
- They were electroporated with very small doses of chemotherapeutic drug, Taxol (9nM) and hormone drug, Tamoxifen, using 200V/cm, 40ms and 1200V/cm, 200µs pulses.

- The impedance results compared with drug only saple, indicate the enhanced conduction of the electroporated samples.
- There is up to 5 orders of magnitude enhanced conduction between the electroporated and the non-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 or low permeable to the drugs.
- The samples followed Schwan's power-law with very close correlation and also exhibited alpha and beta dispersion, indicating the true dielectric dispersive nature of the MCF-7 cells.
- These results correlate very well with those obtained for tissues and by other researchers.

The investigation of the electrical impedance of cancer cells over a frequency range enables the identification of the frequency for optimization of the pulse parameters using their frequency-dependent dielectric behavior which could be used for various applications including pathology, diagnostics, and therapeutics using electrical pulses.

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