Irreversible Electroporation Effects: A Drug-Free Treatment for Cancer

Ming-Jung Heish[1], Therese Salameh[2], Ignacio Camarillo[2], and Raji Sundararajan[3]
[1]Radiological Health Science Dept.
[2]Biological Sciences Dept.
[3]Electrical and Computer Engineering Technology Dept.
Purdue University, West Lafayette, IN, USA
phone: 765 494 6912
e-mail: raji@purdue.edu

***Abstract*—Irreversible electroporation is that which causes permanent permeabilization of the cell membranes and the consequent loss of cell homeostasis, when an electrical field is applied to cancer cells. The advantage this technique is that it is drug-free and is targeted. The purpose of this study is to evaluate the effects of various electroporation parameters on different cancer cell lines, such as estrogen receptor alpha (ERα) positive breast cancer cells MCF-7, ERα negative breast cancer cells MDA-MB-231, and mouse breast cancer cells 4T1. They were treated with various pulse parameters of electroporation, including 100V/cm, 24 ms; 500 V/cm, 1 ms; 500 V/cm, 24ms; 1200 V/cm, 100μs; 1500 V/cm, 300μs; and 1500 V/cm, 2ms. Each parameter had eight pulses with one-second interval in between pulses. The numbers of live and dead cells were determined immediately after electroporation using Trypan Blue staining. The results of this study indicate the differences in the viabilities and hence the aggressiveness of the cell lines experimented and the irreversible electroporation (IRE) parameters needed to be delivered to obtain desired effect on proliferation control and complete tumor ablation.**

I. Introduction

The pitiful response rate of the most commonly used FDA approved breast cancer drugs (Table 1 [1]) indicates that not all patients could get well from current chemo and hormone therapy regimens. There is a critical need for alternate, physical techniques to treat cancers. One promising technique is the use of controlled electrical pulses of appropriate intensity and duration, known as electroporation [2-5], wherein high intensity, short duration pulses are utilized to temporarily open up pores to allow drug molecules to enter, which otherwise are nonpermeable.

The cell membrane separates cell cytoplasm and external environment, acting as a barrier that controls substances coming in and out of the cell. Electrical field pulses can be applied to open the cell membrane transiently, allowing non-permeant large molecules to enter the cell, and this leads to the term electroporation [2]. The mechanism of electroporation is not fully understood yet. The electrical field pulses applied could have changed

the electrical potential across the cell membrane and caused the membrane to change its shape, which can generate nano-scale pores on the membrane [3].

Electroporation can have three effects on the cell membrane: no changes on the cell membrane, temporary open the cell membrane after which cell can still survive (reversible electroporation), and permanently open the cell membrane and the cell dies after (irreversible electroporation) [3].

Reversible electroporation is used in electrogenetherapy and electrochemotherapy, which insert DNA or inject drugs and machomolecules into the targeted area. Electrodes are placed around the tumor area, generating reversible permeabilization that allows substances to pass the cell membrane and into the cytoplasm [3]. Bleomycin and cysplatinum are the most affective drugs for cancer treatment so far. The upper limit to the range of electrical parameters that induce reversible electroporation are used for irreversible electroporation.

Irreversible electroporation (IRE) is that which causes permanent permeabilization of the cell membranes and the consequent loss of cell homeostasis, when an electrical field is applied to cancer cells [3, 6]. Advantages of IRE are that it's a non-thermal mechanism of action, dependent on the blood flow, allows focal tissue ablation, and it requires a short time of application [3]. IRE allows ablation of tumors in close proximity to other tissues. Other ablation mechanisms such as radiofrequency thermal ablation (RFA) release energy to the surrounding tissues and affect the vascularity and connective tissue structure [4]. RFA first uses ultrasound, computed tomography, or magnetic resonance to locate the tumor and then insets a small needle-electrode into the tumor [5]. Ionic vibration at the needle tip generates frictional heat that ablates the tumor.

The focus of this study is to determine the responses of different cancer cells and compare the aggressiveness of those cells. We performed electroporation experiments with three different breast cancer cells. This provides the aggressive level of each cancer and the parameter that is required to obtain desired effect.

TABLE 1: RESPONSE RATES OF METASTATIC BREAST CANCER TO SINGLE DRUG SYSTEMIC THERAPY [1]

Drug	Response Rate
Doxorubicin	25% to 40%
Paclitaxel	17% to 54%
Tamoxifen	21% to 41%

II. MATERIALS AND METHODS

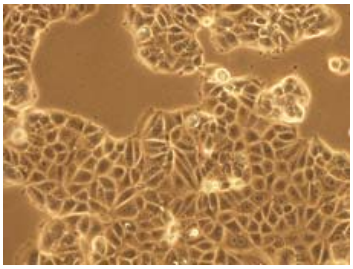
A. Cell Lines

Breast cancer cells MCF-7, MDA-MB-231 and 4T1 were cultured in RPMI-1640 medium with 10% fetal bovine serum and 1% penicillin/streptomycin Stock and incubated in a 5% CO_2 atmosphere at 37°C. Fig. 1 illustrates the cell morphologies [7-9].

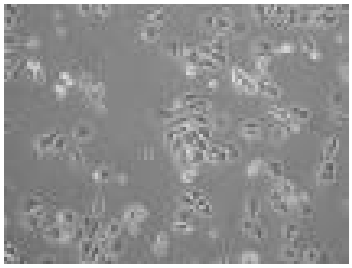
B. Electroporation

Cells were kept in RPMI-1650 medium with 10% fetal bovine serum and 1% penicillin/streptomycin stock and were dissociated from the flasks with 0.25% trypsin/EDTA

solution. The concentrated of cells was determined manually or using a hemocytometer and made to a final concentration of 1 x 10^6 cells/mL. 700 μL and 400 μL of the cell solution were added to 0.4 cm and 0.2 cm cuvettes, respectively. Electroporation was performed using a BTX ECM830 electroporator (Harvard Apparatus, USA) shown in Fig. 2. Cells were treated with various pulse parameters including those shown in Table 2, which had 8 pulses each with 1second interval in between pulses.



(a) MCF-7 Cells



(b) MDA-MB-231 Cells

(c) T41 Cells

Fig. 1. Breast Cancer Cells Studied for Irreversible Electroporation.

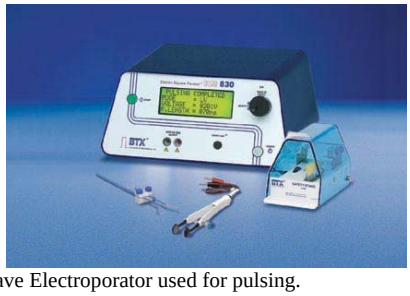


Fig. 2. BTX ECM830 Square Wave Electroporator used for pulsing.

C. Viability Calculations

After electroporation, cell viability was determined by mixing 10 μL of cells from the cuvettes with 90 μL of Trypan Blue solution. The mixture was placed in a hemocytometer or under a microscope. Trypan Blue can only penetrate dead cells, so cells that were dyed blue are dead.

TABLE 2: TREATMENT PARAMETERS

Electric Field Intensity, V/cm	Pulse Width	# Pulses
Control	-	-
100	24ms	8
500	1ms	8
500	24ms	8
1200	100μs	8
1500	300μs	8
1500	2ms	8

III. RESULTS AND ANALYSIS

Figures 3-6 summarize the results of three cancer cell lines that underwent the same electroporation parameters. We performed two different experiments for each set of experimental parameters. For MCF-7 and MDA-MB-231 cells, the viabilities were determined through hemocytometer. For 4T1, the number of live cells was counted manually under a microscope.

Figures 2 and 3 show the viabilities of MCF-7 and MDA-MB-231 cells. The viabilities of MCF-7 are mostly higher than 90%, except for two of the six parameters. Under the parameter 500V/cm, 24ms, 80% of MCF-7 cells survived. The parameter 1500V/cm, 2 ms reduced the viability to 38%. Previous work by Miller, Leor, and Rubinsky had different results from ours [3]. In their paper, two of their parameters were similar to ours, which were 1500V/cm, 3ms and 500V/cm, 24ms. The viabilities for these parameters were close to 20%. For 1500V/cm, our data is higher because the duration was 2 ms instead of 3ms. For MDA-MB-231 cells, the viabilities are also in the 90% range and are higher than those of MCF-7. For the parameter 1500V/cm, 2ms, that reduced to 80.2%. Comparing MCF-7 with MDA, we can see that the parameters that set the two cancer cell lines apart are 500 V/cm, 24 ms and 1500V/cm, 2ms. We can conclude that MDA is a more aggressive cancer cell than MCF-7 and requires a higher voltage and longer duration to have the same effect as MCF-7. Fig. 5 shows a comparison of these two cell lines.

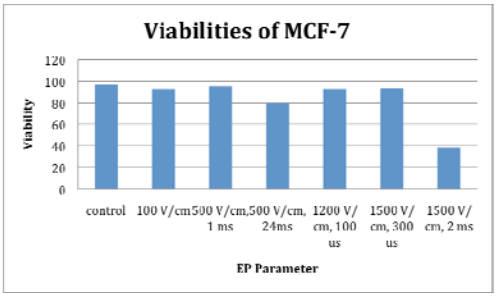


Fig. 3. Viability of MCF-7 cells after treatments with various pulse parameters.

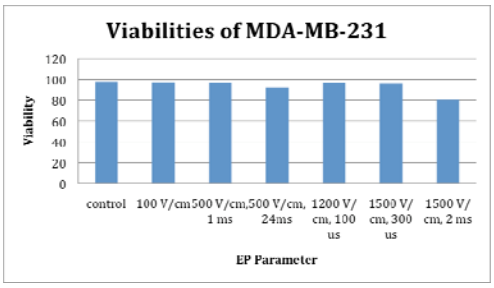


Fig. 4. Viability of MDA-MB-231 cells after treatments with various pulse parameters.

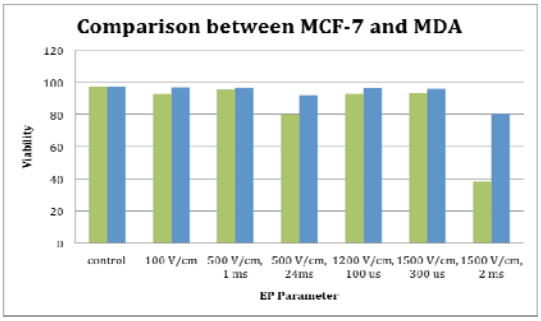


Fig. 5. Comparison of Viabilities of MCF-7 and MDA-MB-231 cells after treatments with various pulse parameters.

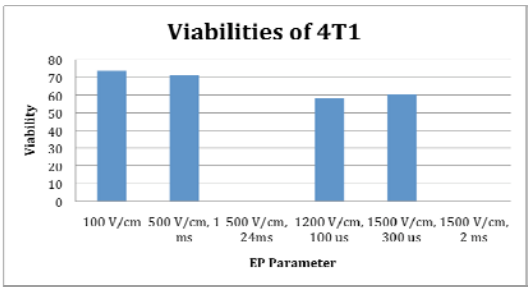


Fig. 6. Viability of T41 cells after treatments with various pulse parameters.

Figure 6 shows the responses of 4T1 cells. The viabilities spread into a wider range than MCF-7 and MDA. The highest viability was 73.8% and the lowest was 0%. There were two parameters that killed all the cells, which are 500 V/cm, 24 ms and 1500 V/cm, 2 ms. This result shows that longer duration is more effective towards permeabilization in 4T1.

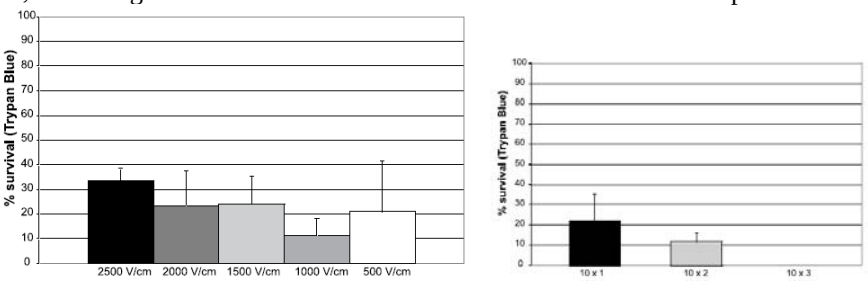
IV. Discussion And Conclusions

We have examined the efficacies of drug-free irreversible electroporation in two human breast cancer cell lines exhibiting highly different phenotypes, and a murine (mouse) breast cancer cell line. These are the estrogen receptor alpha (ERalpha) - positive, weak-

ly invasive, luminal epithelial-like MCF-7, the ERalpha negative, highly invasive, fibroblast-like MDA-MB-231 cell lines [10], and the 4T1 metastatic breast cancer model which is an excellent mouse model for the study of metastatic progression of breast cancer in humans [11] respectively. MCF-7 is a widely used epithelial cancer cell line, derived from breast adenocarcinoma. MCF7 cells retain characteristics of differentiated mammary epithelium, including ability to process estradiol via cytoplasmic estrogen receptors. Although easy to propagate, the cells are generally slow-growing.

Our results show the promise of completely ablating the tumors using electrical pulses of both high and low intensities of micro and millisecond durations. There were complete cell deaths in the case of 4T1 cells at 500V/cm, 24ms, 8 pulses as well as 1500V/cm, 2ms, 8 pulses. The corresponding viabilities for MCF-7 and MDA-MB-231 for the same pulse parameters are 38% and 80.2% respectively. These results indicate the difference between the two human breast cancer cell lines and the mouse breast cancer cell line. The difference in the viabilities of these various cells could be due to the difference in the cell morphology, type of cell and the membrane. The considerable differences in response to protein kinases between the two human cell lines, MCF-7 and MDA-MB-231 is elucidated in a previous publication [10].

A comparison with previous research indicate similar trend of results while the magnitudes of viability vary for a given intensity. Fig. 7 shows the results obtained by Rubinsky team [3]. They applied single pulse (Fig. 7a) and trains of 10 pulses (Fig. 5b) and the cell line used was human hepatocarcinoma (HepG2) which is obtained from liver hepatocellular carcinoma. It can be seen that using 30 (10x3) pulses at 1500V/cm, 300µs pulses, there was complete cell death. This indicates that we could completely kill the cells using series of electrical pulses (Fig. 7b). When we repeated 1500V/cm, 300µs, 30 pulse parameters, using MCF-7 human breast cancer cells, there was notable viability (data not shown). We did not observe complete cell death. We could observe complete cell death using longer (ms) pulses at various intensities, including 500V/cm, and 1ms pulses, indicating the differences in the cell sensitivities to the electrical pusles.



(a) (b)

Fig. 7. Viability of cells after Irreversible Electroporation – a) for various pulse parameters using single pulse, the durations of which are: 1ms, 1.5ms, 3ms, 6ms, and 24ms, b) due to a number of pulses for 1500V/cm, 300µs pulses [6].

The outcome also depends on the number of pulses and the interval between the pulse trains. In the case of trains of pulses, such as 10 pulse trains, three times to obtain 30

pulses (3x10), the cell death or the viability depends on the interval between the each train of 10 pulses.

Our results indicate the promise potential of this treatment for transfer to clinical applications eventually. Fig. 8 shows an application of microsecond pulse irreversible electroporation for cancer in a human organ. It can be applicable to cancer in the liver, kidney, and other sensitive organs without exposing the patient to traditional invasive surgical procedures, harsh chemotherapies, or radiation treatments that might also damage nearby healthy areas [12].

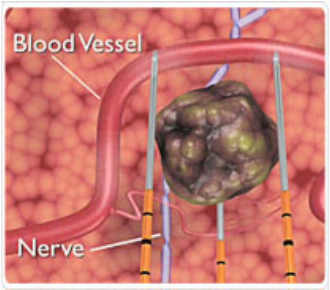


Fig. 7. Medical application of irreversible electroporation for treating an organ with cancer.

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