

CANCER CELL VIABILITY AND SURVIVAL *IN VITRO* AS FUNCTION OF CELL SURFACE ELECTRIC CHARGE: AN ANALYTICAL STUDY

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ABSTRACT

Electrical charge is an indicator of the cellular state of health. In cells, the cell membrane is a leaky dielectric. Capacitors are composed of two conducting sheets separated by a thin layer of insulating material known as a dielectric. Cells contain several forms of biological capacitors, which consist of an insulating material (the membrane) covered on both sides by collection of charged dissolved minerals, which serve the function similar to a conducting metal plate. This means that any condition, illness or change in dietary intake that affects the composition of the cell membranes and their associated minerals can affect and alter cellular capacitance. Healthy cells have a higher whole cell and cytoplasm conductivity and higher membrane capacitance than the malignant cells. The electric charge of cell membranes of mammals is negative at physiological pH. It is well-known that surface of cancer and tumor cells carry negative charge in excess to that of normal cells which is responsible for their increased mobility and invasiveness. The high negative surface charge on malignant cells and trophoblasts may mediate the lack of immune rejection of these cells. Thus, neutralizing or removing the tumor cell negative charge may allow negatively charged lymphocytes to approach and destroy them and thereby, the viability and survival of the cancer cells may be affected at least *in vitro*. The present study serves to test this hypothesis on invasive human breast carcinoma cell line MDA-MB-231 (ER⁻) using specially designed and constructed combination of circuits. Preliminary results showed that out of the 8 designed circuits, sets 1, 2 and 7 were able to produce significant growth inhibition of MDA cells *in vitro*. The application of electrostatic field through specially designed circuits is unique and has never been reported previously. Our long-term goal is to develop a minimally invasive device that will selectively target and destroy both metastatic and non-metastatic cancer cells in humans. Better understanding of effect of decreasing surface charge density of tumor cells may lead to device effective treatment strategy of human tumors in future.

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INTRODUCTION

Cell membranes are composed of a bilayer of highly mobile lipid molecules that electrically behave as an insulator (dielectric). The insulating property of the cell membrane also restricts the movement of charged ions(1-4) and electrons across the membrane except through specialized membrane spanning protein ion channels respectively (5-8). Thus, the lipid structure of a cell membrane makes it relatively impermeable to the passage of charged molecules. As a result, cell membrane behaves like a leaky dielectric.

Further, illness or change in the dietary intake affects the composition of the cell membranes and their associated

minerals alter the cellular capacitance, capacitance relaxation and electrostrictive energy (9-11). The outermost electrically negative zone of a cell membrane is composed of negatively charged sialic acid molecules that cap the tips of glycoprotein and glycolipids. The negative zone is separated from the positive cell membrane surface by a distance of about 20 μm . This outermost calyx zone of steady negativity makes each cell a negatively charged body. As a result each cell creates a negatively charged field around itself influencing other charged bodies close to it(12).

Because the external cell membrane and the membranes of cell organelles like the mitochondria in

animals and the chloroplasts in plants are biological capacitors, they have the capacity to accumulate and store charge and hence energy, to be given up when needed. Since energy is needed to run any type of machinery be it mechanical or biological, it makes sense that those nutrients that can enhance energy production and energy storage can have profound biological effects.

When cell membranes are damaged by free radicals their ability to hold an electrical charge (capacitance) and to transport minerals and other nutrients is disrupted. When mitochondria are damaged, the cell's ability to make energy is impaired. When the genetic code is damaged, cells cannot reproduce normal cells. Free radicals also cause lipid peroxidation, which can result in lowering High Density Lipoprotein cholesterol and damage to the cell membranes lining blood vessels. When the delicate membranes lining blood vessels are damaged, an inflammatory process may result which leads to thickening of blood vessels and arterial plaque. The tissue reactions created by free radicals are now thought to be involved in premature aging, cancer, atherosclerosis, arthritis, immune disorders and other degenerative diseases.

The electric charge of cell membranes of mammals is negative at physiological pH (13-14). Any perturbation in the action of the cell is manifested by variations in the action of the cell membrane, that is, in its electric layer. Cell membrane charge increases during tumorigenesis and decreases during necrosis (15). Determining the electric charge of the membrane as a function of environmental pH, acid (CTA) and basic (CTB) functional group concentrations and their average association constants with hydrogen (KAH) or hydroxyl (KBOH) ions allows monitoring of changes caused by cancer transformation (16).

It is well known that malignant neoplastic cells are different in their surface properties from their normal counterparts. Unusual cell-to-cell interactions in malignant cells are the most important behavior for distinguishing them from their normal counterparts and for determining the prognosis of patients suffering from cancer.

Cancerous cell lines are an excellent means for examining the mechanisms of carcinogenesis, and research using *in vitro* cultures is bringing significant benefit. Therefore, the purpose of this work was to determine the effect of electrical properties of membranes of human breast cancer cells (MDA-MB-231) on their viability *in vitro*.

MATERIALS AND METHODS

Biological evaluation

Reagents

0.25% Trypsin-EDTA (1X), DMEM/F-12 (1X) (Dulbecco's Minimum Essential Medium) and Antibiotic (100X) were obtained from Gibco, Life Technologies, whereas FBS and MTT were from Himedia. All analytical grade chemicals were used in the study.

Cell line

MDA-MB-231 (human breast carcinoma, ER, tumorigenic and invasive cell line) was obtained from the National Centre for Cell Science (NCCS), Pune, India, and as such, was maintained by sub-culturing and passaging as monolayers in 25 and 75 cm² cell culture flasks (Nest, Tarsons) at 37°C in Tissue and Cell Culture Lab, Era's Medical College, Lucknow, in an incubator gassed with an atmosphere of 5% CO₂ at 95% humidity, in advanced Dulbecco's Minimum Essential Medium (DMEM) containing phenol red as a pH indicator and supplemented with 5% FBS. The medium, prior to be used in cell culture experiments was vacuum filtered using a Corning filtration system. The medium requires an atmosphere of 5% CO₂ to produce HCO₃ buffering capacity to maintain pH at 7.4 for normal cell growth.

Experimental setup

For experiments, cells were trypsinized and cultured in 25 cm² culture flasks (1.0 x 10⁵ cells/flask). Eight combinations of capacitors were set up in flasks named as experimentals whereas matched flasks served as control (medium + cells only) for each combination. The controls served to negate any charge contributed by the culture medium. The following were the combinations:

Set 1:

1. Control: Medium + cells

2. **Experimental:** Positive terminal of wire inside the flask (through capacitor) and negative terminal earthed

Set 2:

1. Control: Medium + cells

2. **Experimental:** Both plates of capacitor connected to two needles through wires

Set 3:

1. Control: Medium + cells

2. **Experimental:** Flask containing medium and cells with two stainless steel needles connected to one plate of capacitor, the other connected to battery.

Set 4:**1. Control: Medium + cells**

2. Flask containing medium and cells with one stainless steel needle connected to one plate (+) of capacitor, the other (-) earthed

Set 5:**1. Control: Medium + cells**

2. Flask containing medium and cells with three copper wires connected to one plate (+) of capacitor, the other (-) earthed

Set 6:**1. Control: Medium + cells**

2. Flask containing medium and cells with one copper wire connected to (+) of battery, the other (-) earthed

Set 7:**1. Control: Medium + cells**

2. Flask containing medium and cells with two copper wires (unpolished) connected to one plate (+) of capacitor, the other (-) earthed.

Set 8:**1. Control: Medium + cells**

2. Both plates of capacitor connected to two needles

MORPHOLOGICAL STUDY

For morphological analysis, cells in 25 cm² culture flasks were observed under phase contrast microscope and photographed (Nikon Eclipse Ti, Japan).

CELL VIABILITY ANALYSIS

For Cell Viability Analysis, the cells from all wells were trypsinized, resuspended in culture medium and read in a Tali Image-Based Cytometer, Life Technologies (Invitrogen). The number of live and dead cells in experimental and control wells was determined. The number of live cells in both treated and control wells was used for calculating the percentage cytotoxicity as % Cytotoxicity = $\frac{\text{Live Cell No. in Treated Wells} - \text{Live Cell No. in Control Wells}}{\text{Live Cell No. in Control Wells}} \times 100$.

Statistical Analysis:

Results were expressed as mean \pm standard deviation (SD) of experiments done in triplicates.

Table 1 shows the cytotoxic effects of various combinations on the viability of MDA cells *in vitro*

RESULTS

S. No.	Flask	Seeding Density (cells/ml)	No. of Live Cells	% Cytotoxicity
Set 1	Control	5×10^4	$7.05 \times 10^5 \pm 12.34$	79.9
	Experimental	5×10^4	$1.42 \times 10^5 \pm 20.22$	
Set 2	Control	1×10^5	$1.29 \times 10^6 \pm 9.02$	75.7
	Experimental	1×10^5	$3.14 \times 10^5 \pm 12.54$	
Set 3	Control	1×10^5	$1.0 \times 10^6 \pm 19.01$	Nil
	Experimental	1×10^5	$1.05 \times 10^6 \pm 21.09$	
Set 4	Control	1×10^5	$7.06 \times 10^5 \pm 10.02$	32.6
	Experimental	1×10^5	$4.76 \times 10^5 \pm 15.03$	

Set 5	Control	1×10^5	$1.16 \times 10^6 \pm 12.06$	Nil
	Experimental	1×10^5	$1.27 \times 10^6 \pm 23.12$	
Set 6	Control	1×10^5	$7.06 \times 10^5 \pm 19.02$	Nil
	Experimental	$1 \times 10^5 \pm$	$7.92 \times 10^5 \pm 24.05$	
Set 7	Control	1×10^5	$1.25 \times 10^6 \pm 5.06$	99.8
	Experimental	1×10^5	$0.24 \times 10^4 \pm 8.01$	
Set 8	Control	1×10^5	$1.05 \times 10^6 \pm 2.04$	Nil
	Experimental	1×10^5	$1.01 \times 10^6 \pm 4.86$	

Table 1: Cytotoxic effects of various sets on MDA cells after 96 h.

Set 1: Positive terminal of wire inside the flask (through capacitor) and negative terminal earthed.

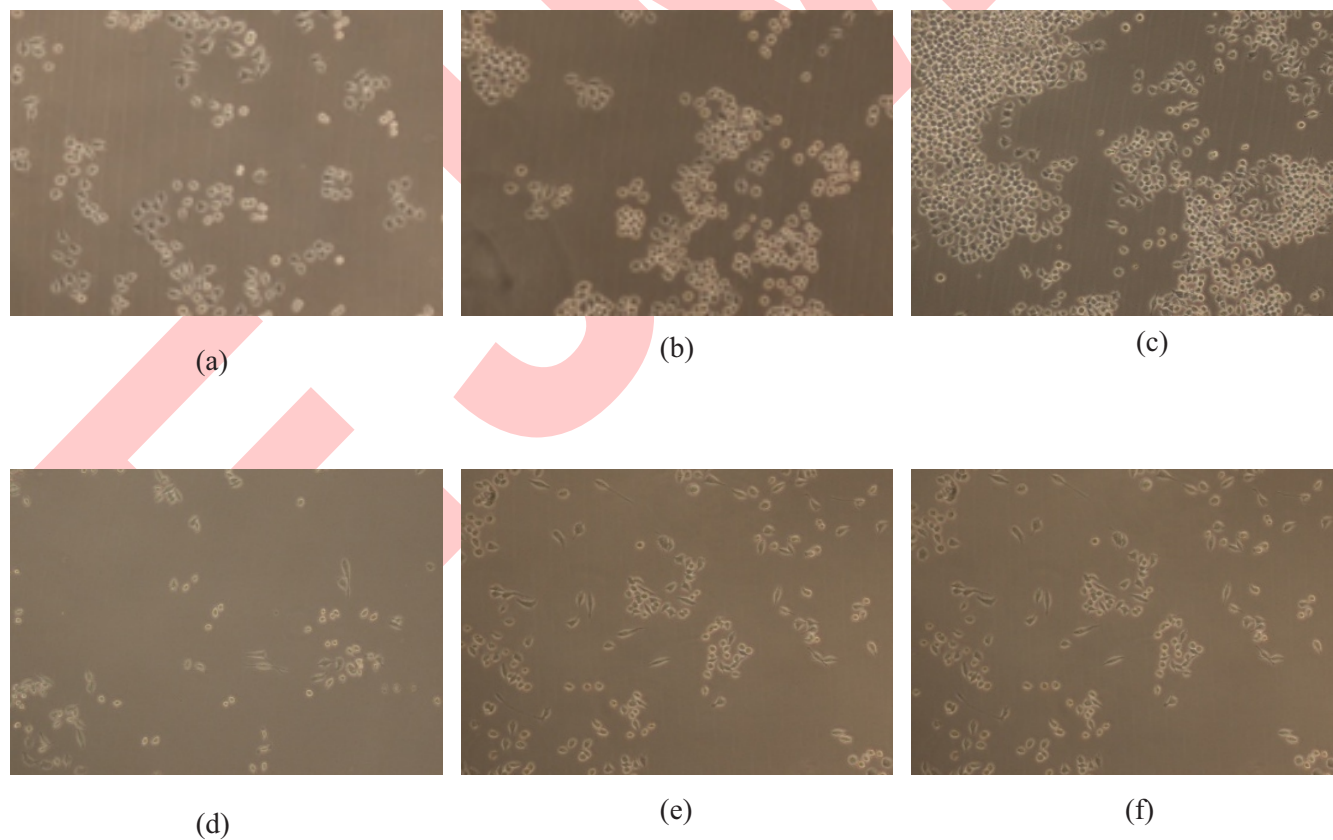


Fig. 1 (a-c) Controls showing MDA human breast cancer cells at 48, 72 and 96 h respectively (d-f) Effect of Set 1 on MDA cells at 48, 72 and 96 h respectively (Magnification 10X).

Set 2: Both plates of capacitor connected to two needles through wires

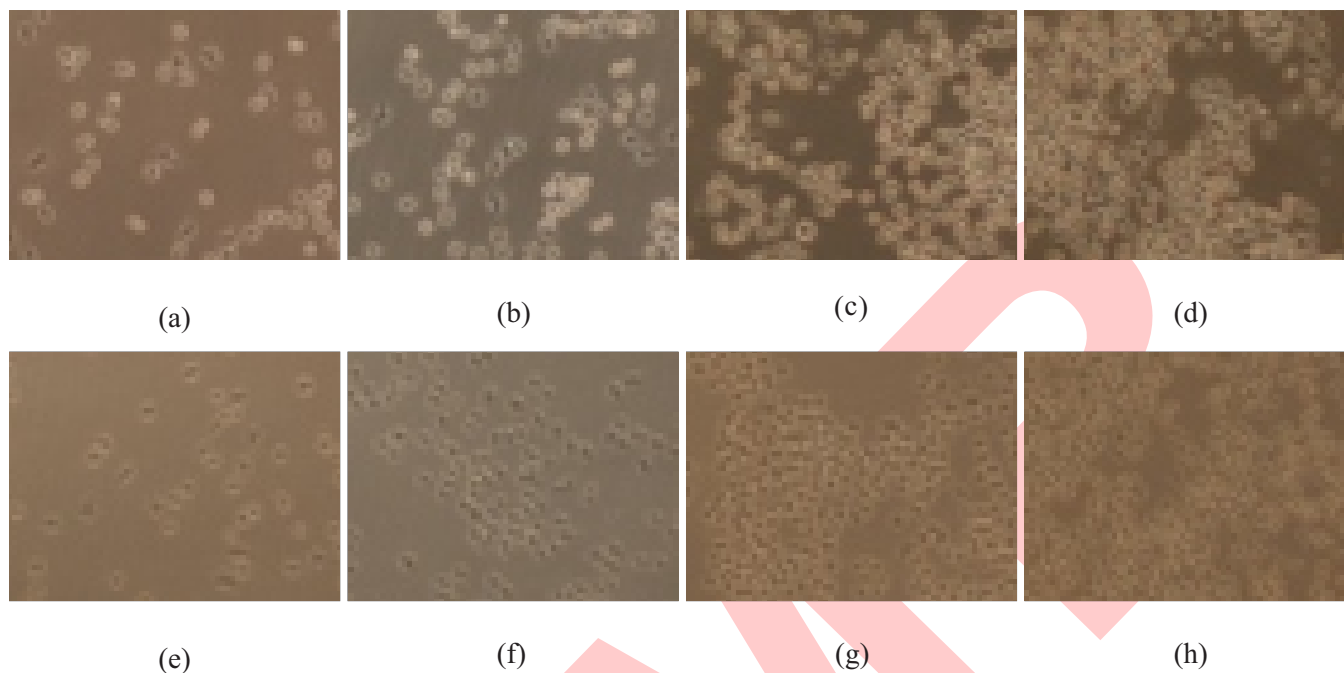


Fig. 2 (a-d) Controls showing MDA human breast cancer cells at 24, 48, 72 and 96 h respectively (e-h) Effect of Set 2 on MDA cells at 24, 48, 72 and 96 h respectively (Magnification 10X).

Set 3: Two stainless steel needles connected to one plate of capacitor, the other connected to battery.

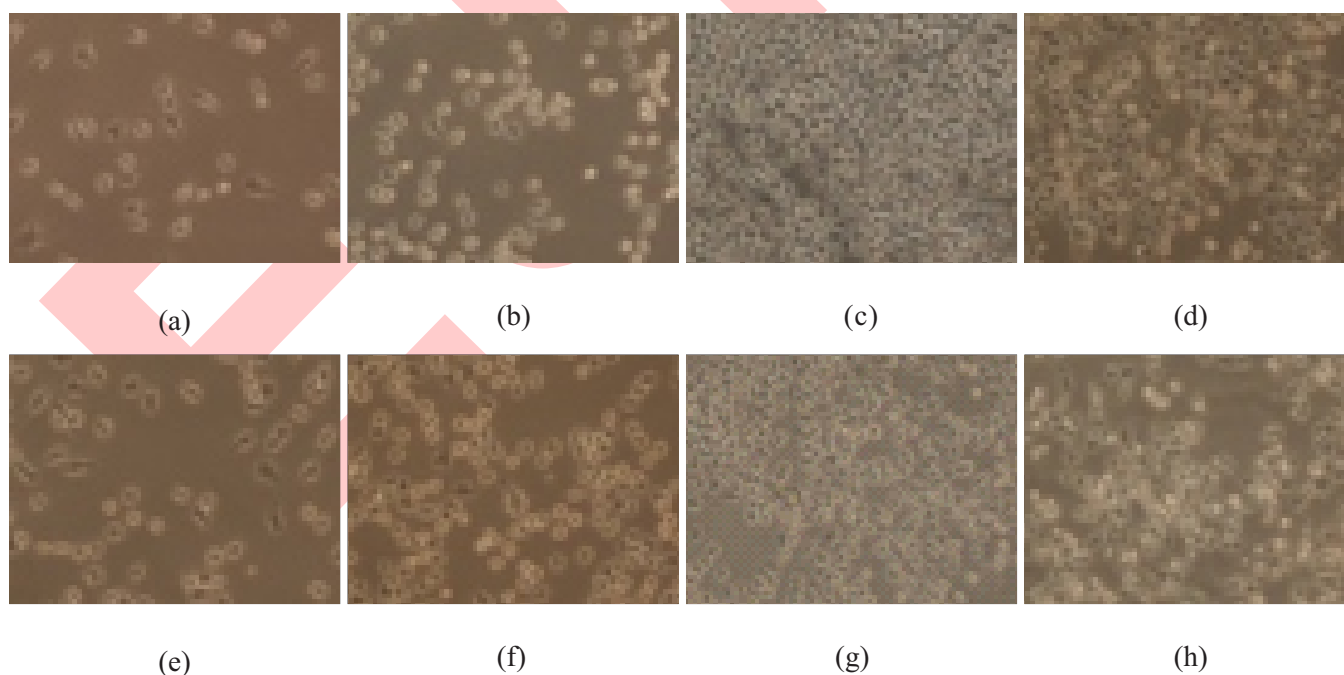


Fig. 3 (a-d) Controls showing MDA human breast cancer cells at 24, 48, 72 and 96 h respectively (e-h) Effect of Set 3 on MDA cells at 24, 48, 72 and 96 h respectively (Magnification 10X).

Set 4: Flask containing medium and cells with one stainless steel needle connected to one plate (+) of capacitor, the other (-) earthed

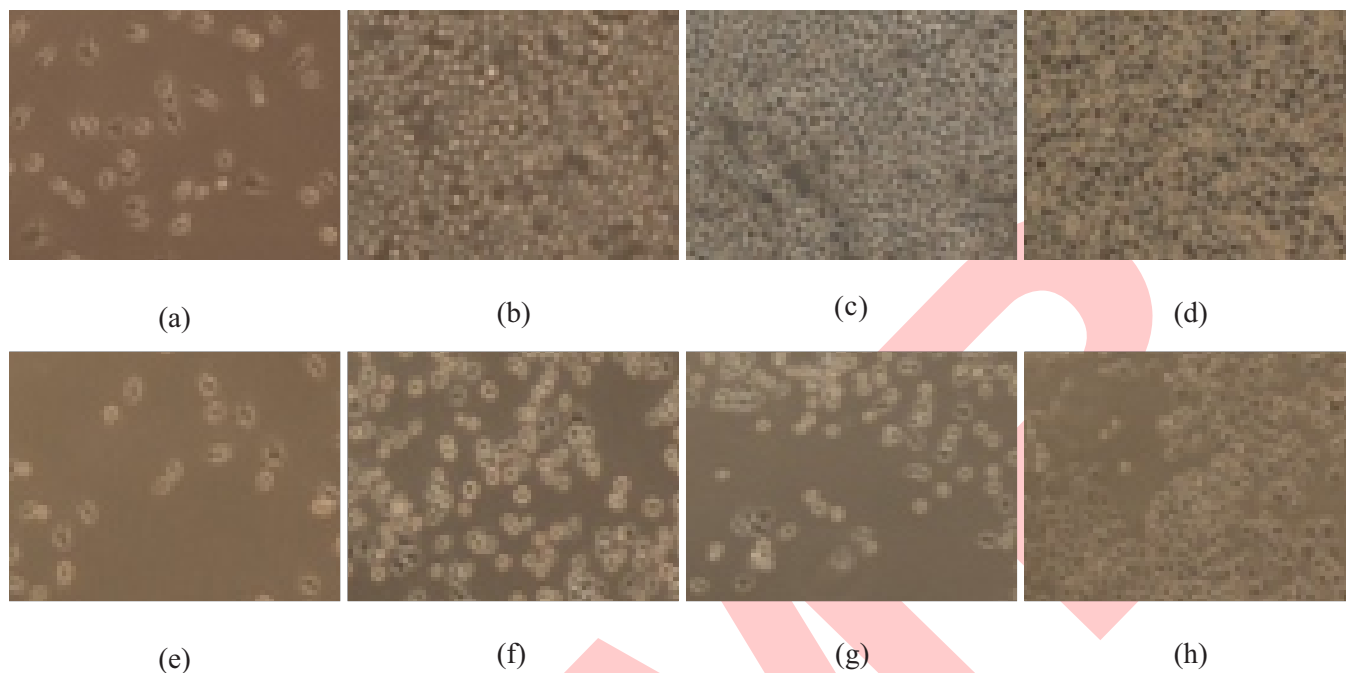


Fig. 4 (a-d) Controls showing MDA human breast cancer cells at 24, 48, 72 and 96 h respectively: (e-h) Effect of Set 4 on MDA cells at 24, 48, 72 and 96 h respectively (Magnification 10X).

Set 5: Flask containing medium and cells with three copper wires connected to one plate (+) of capacitor, the other (-) earthed

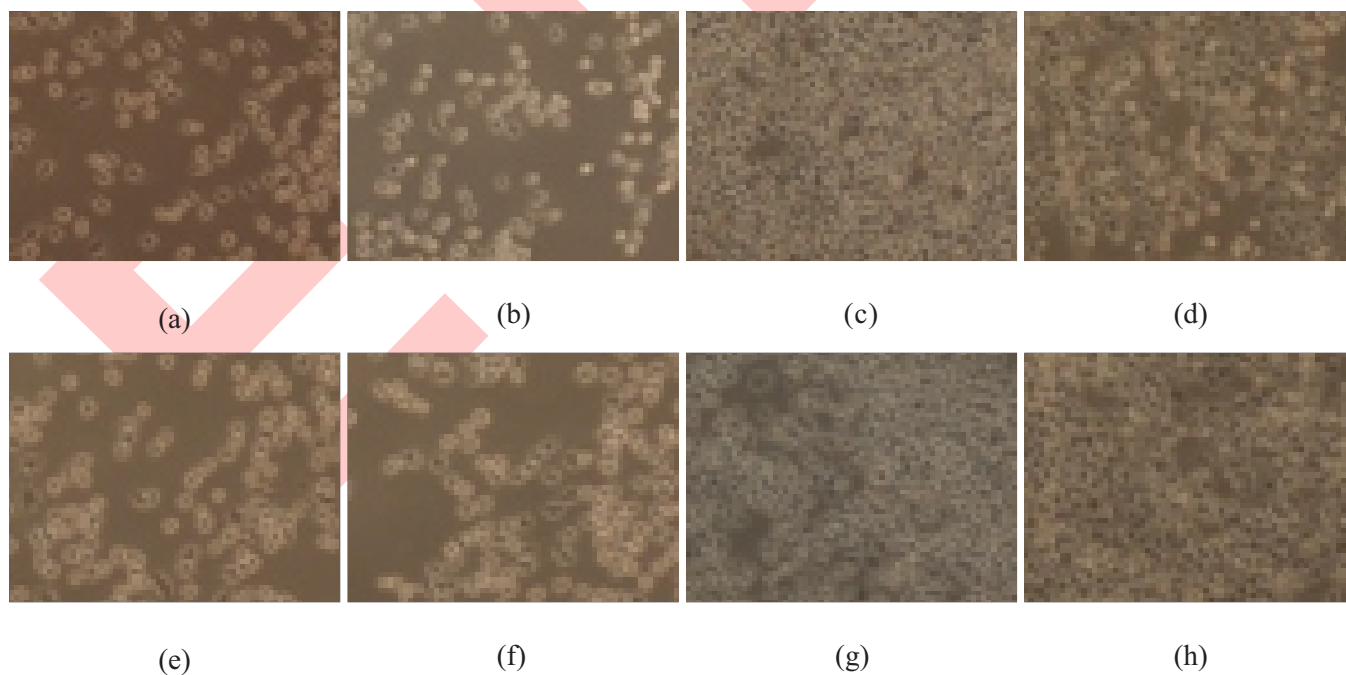


Fig. 5 (a-d) Controls showing MDA human breast cancer cells at 24, 48, 72 and 96 h respectively: (e-h) Effect of Set 5 on MDA cells at 24, 48, 72 and 96 h respectively (Magnification 10X).

Set 6: Medium and cells with one stainless steel needle connected to one plate (+) of capacitor, the other (-) earthed

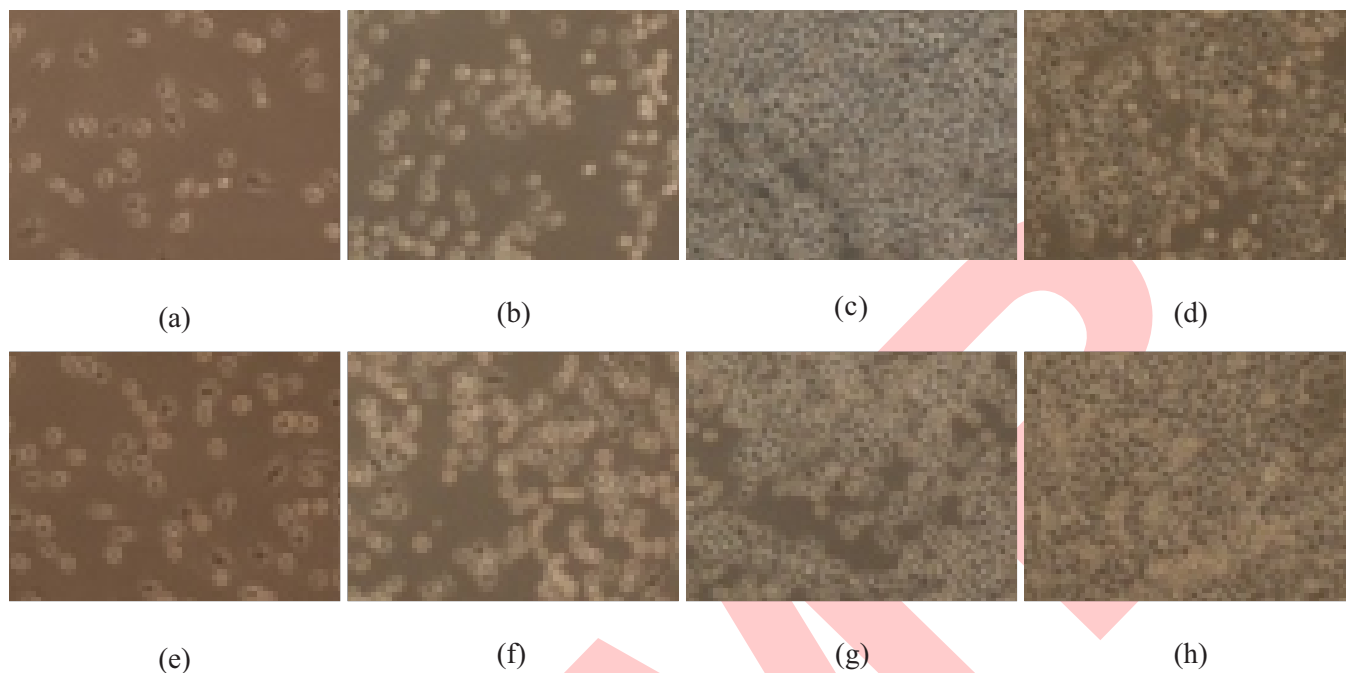


Fig. 6 (a-d) Controls showing MDA human breast cancer cells at 24, 48, 72 and 96 h respectively: (e-h) Effect of Set 6 on MDA cells at 24, 48, 72 and 96 h respectively (Magnification 10X).

Set 7: Medium and cells with two copper wires (unpolished) connected to one plate (+) of capacitor, the other (-) earthed

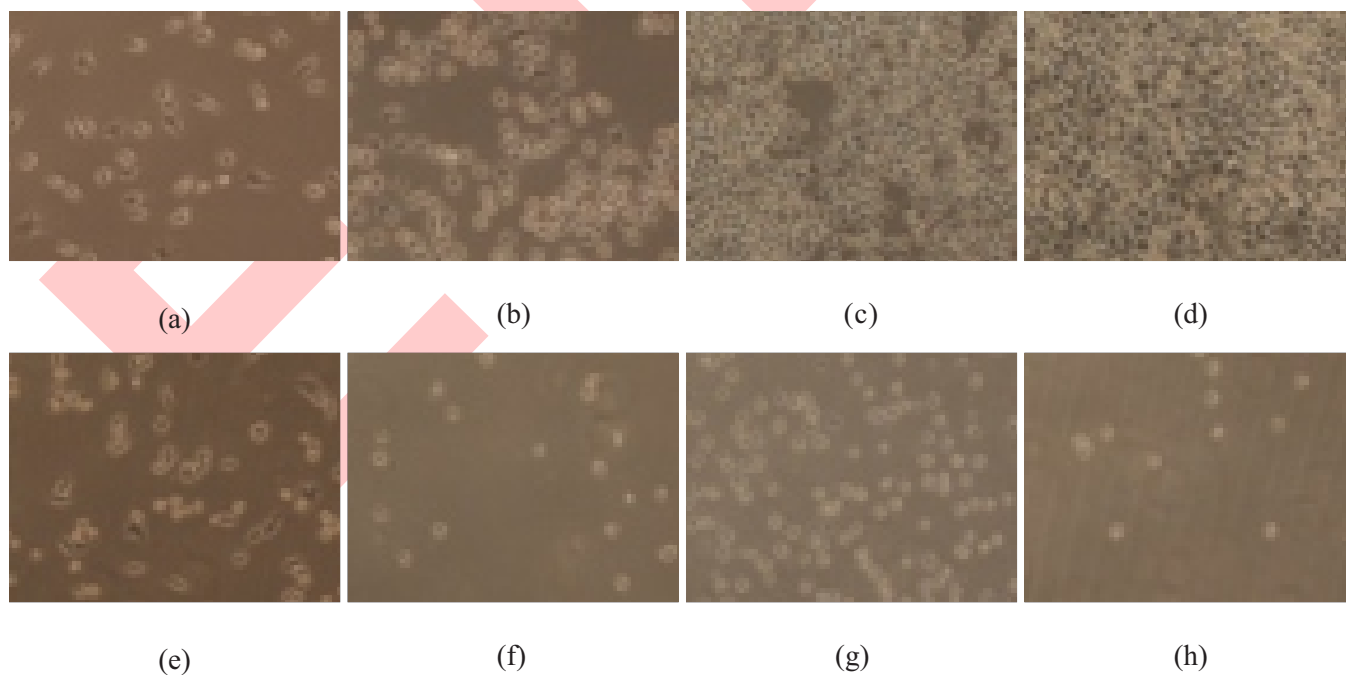


Fig. 7 (a-d) Controls showing MDA human breast cancer cells at 24, 48, 72 and 96 h respectively: (e-h) Effect of Set 7 on MDA cells at 24, 48, 72 and 96 h respectively (Magnification 10X).

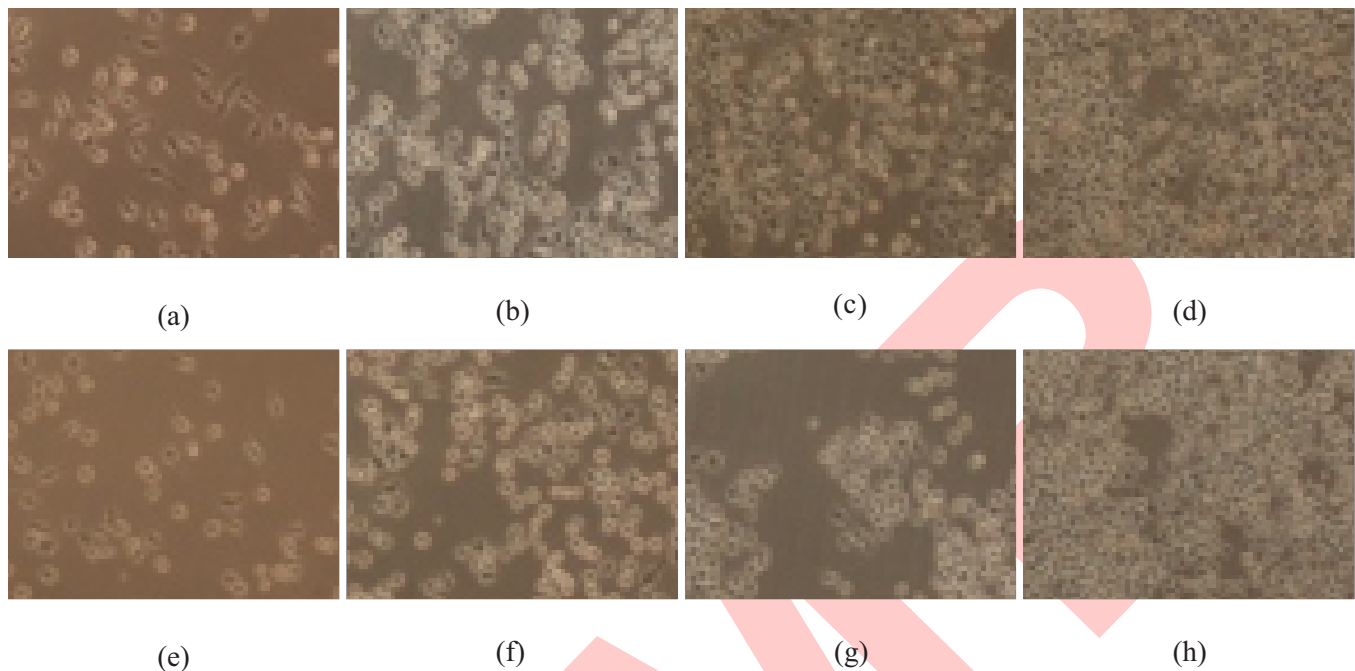
Set 8: Both plates of capacitor connected to two needles

Fig. 8 (a-d) Controls showing MDA human breast cancer cells at 24, 48, 72 and 96 h respectively: (e-h) Effect of Set 8 on MDA cells at 24, 48, 72 and 96 h respectively (Magnification 10X).

after 96 h. While set 1, 2 and 7 caused significant cytotoxicity in MDA cells, set 4 was not as effective in killing MDA cells *in vitro*, whereas sets 3, 5, 6 and 8 did not cause any cell death in culture after 96 h of observation. Figs. 1-8 respectively depict the cytotoxic and morphological analysis of MDA cells in absence (a-d) and presence (e-h) of the designed circuits/sets. Results are mean \pm SD of experiments done in triplicates.

DISCUSSION

Electromagnetic fields continue to find useful applications in human health. In the past decade, there has been a resurgence of interest in applying electromagnetic fields for cancer treatment. Some results have been encouraging. For example, specific combinations of ultra-high frequency fields and electrophoresis result in epithelial tumor regression and dissolution(17-21). Low power alternating fields, in kilohertz frequency range, are reported to destroy cancer cells during mitosis (22-24). Finally, electromagnetic fields can be selectively tuned to target cancer cells making them more susceptible to chemotherapeutic agents. In the current study, we propose a Phase I study to explore the effects of specially designed and constructed combination of circuits on human mammary cancer cell cultures with the objective of identifying parameters that may render cancer cells non-viable and/or increase their sensitivity to chemotherapy drugs.

All cells exhibit an electrical potential across their membranes called a membrane potential (MP). Several lines of investigation point to differences in electrical properties between normal and cancerous cells. Several tumor lines have low-resting membrane potentials. A few comparisons have been made between normal and tumor cells within the same tissue cell type.

All healthy living cells have a MP of about -60 to -100mV. The negative sign of the membrane potential indicates that the inside surface of the cell membrane is relatively more negative than the immediate exterior surface of the cell membrane,(25) whereas, cancer cells exhibit both lower electrical membrane potentials and lower electrical impedance than normal cells(26-28). The excessive amount of negative charge on the exterior surface of the cell responsible for carcinogenic change and genetic change results from development of cellular electrical abnormalities(29). Also the depolarization (fall in membrane potential) of the cancer cell membrane due to the accumulation of excess negative surface charges may precede and create the reduction in intracellular potassium and the rise in the intracellular sodium ions(29-30). The cancer cells have significantly more sialic acid molecules in their cell coat and as a result cancer cells have a greater surface negativity. It has been found that the cells become more electronegative in the course of

cancerization. Membrane degeneration occurs in the initial phase of carcinogenesis first in the external cell membrane and then in the inner mitochondrial membrane and these degenerative changes in the surface membrane causes these membranes to become more permeable to water-soluble substances so that potassium, magnesium and calcium migrate from the cells and sodium and water accumulate in the cell interior(30). Two of the most outstanding electrical features of cancer cells are that they constantly maintain their MP at a low value and their intracellular concentration of sodium is of high magnitude(31-33). This sustained elevation of intracellular sodium may act as a mitotic trigger causing cells to go into cell division (mitosis) (33). In the resting phase normal cells maintain a high MP of around -60 to -100 mV, but when cells begin cell division and DNA synthesis, the MP falls to around 15 mV (25).

A related technique called as electrochemical therapy (EChT) has shown promising results in mice with implanted Jensen sarcoma tumors(34). However, the clinical application of this modality was initiated by the Swedish radiologist Nordenstrom. In 1983, he published a book in which he described his theory of biologically closed electrical circuits and the results of 2 decades of research on EChT treatment of malignancies in animals based on this(35). He also reported the results of EChT in 20 lung cancer patients with 26 tumors in which follow-up after 2 to 5 years revealed that 12 tumors had either disappeared or were markedly reduced in size. This study has stimulated interest in utilizing EChT for treating lung malignancies, and Japanese researchers have subsequently confirmed Nordenstrom's results in animals and in several patients(36-40). Anyway, the real application of the technique widely has begun in China (China-Japan Friendship Hospital as the center of this application), after it was introduced to the country in 1987. Electrodes made of platinum, are inserted into tumor and connecting them with an apparatus, the current arouses strong chemical reactions around electrodes and leads degeneration and necrosis of tumor cells. It is a new method to treat tumor without surgical resection. The final result is caused by current inducing chemical reactions; hence it is called as 'EChT'. The advantages of EChT are that it is much safer, easier to administer, less costly than surgical procedures, and can be just as effective in certain instances. In addition, it provides an opportunity to treat tumors in those patients in whom surgery, radiation, and/or chemotherapy has not been successful or may be contraindicated.

CONCLUSION

To our knowledge, our experimental designs test, for the first time, the killing efficiency of specially designed

circuits on malignant cells *in vitro* carried out under non-hyperthermic conditions (37°C). In future, the hypothesis that non-malignant cells would only be marginally affected by these circuits, would be tested *in-vitro*; that would eventually lead to development of a device effective treatment strategy against human tumors in future.

AUTHORS' CONTRIBUTIONS

MAK conceived experiments, study design and formulated the circuits, ANS coordinated the overall study, whereas RA carried out experiments, analyzed and interpreted data and drafted the manuscript. All authors read and approved the final manuscript.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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