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Inhibiting MCF-7 Breast Cancer Cells Using Low Energy Focused Ultrasound

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Abstract: Inhibiting MCF-7 breast cancer cells using low energy focused ultrasound was demonstrated. Low energy ultrasound wave is applied in the 96-microwell plates containing MCF-7 breast cancer cells through a three-pin line powered by an arbitrary waveform generator. The arbitrary waveform generator was set to emit a 3MHz carrier signal modulated at three resonant frequencies (i.e. 875.71 Hz, 663.16 Hz, and 423.75 Hz) which are derived from three specific target genes of MCF-7 breast cancer cell lines that were identified as mutated genes (i.e. Amphiphysin (AMPH), Fox head Box O3 (FOXO3), and Cell cycle division 25A (CDC25A), respectively). Three different exposure times (i.e. 3, 6, and 9 minutes) were set to determine the inhibiting effect in cell growth. MCF-7 cancer cell line was cultured and divided into four groups to separate the control group with the treated group. After treatment, cytotoxicity test was done to highlight if MCF-7 breast cancer cells are still viable after low energy ultrasound treatment. Absorbance values were taken from the spectrophotometer read-outs to compare the effect of the ultrasound treatment with the control group. T-test has shown that low energy ultrasound treatment has significant effect on MCF-7 breast cancer cells except for AMPH resonant frequency within 6-minutes. Statistical result has indicated that the most significant effect on MCF-7 breast cancer cell's demise is when it is exposed to 3 minutes at any of the given frequency. FOXO3's resonant frequency at 423.75 Hz has shown the most significant effect in debilitating MCF-7 breast cancer cell growth. This suggests that the low energy focused ultrasound can be an effective method against the proliferation of cancer cells.

Keywords: MCF-7 breast cancer cells**,** low energy focused ultrasound

I. INTRODUCTION

The Philippine Society of Medical Oncology reported that breast cancer is one of the most common type of cancer that has been diagnosed with incidence at 16% of 80,000 or simply put 12,800 in the Philippines alone [1]. Most of the common factors of getting breast cancer are family history of breast cancer, excessive consumption of alcohol, being overweight, and early menstruation [2]. Some of the most common cancer therapies being done for breast cancer would involve surgery, chemotherapy, radiation therapy, and targeted therapy. These might inhibit cancer cell growth but it is also accompanied with physical side effects to the body.

Aside from the existing cancer treatment that is available in the market, another tool is being investigated presently because of its potential to treat cancer, which is the ultrasound waves. There is already a review for low-intensity ultrasound and the literatures cited in this review shows significant results [3]. Moreover, Guan and Xu (2016) evaluated the use of high-intensity focused ultrasound to breast cancer and it suggests that it can destroy proliferating cancer cells [4]. It has also been incorporated to drug delivery studies through nanotechnology [5].

The researchers designed and built an alternate treatment plan for breast cancer that is patient-

friendly, cost effective, and has minimal side effects. Low energy focused ultrasound was used because if its low form factor, low cost, and readily available electronic component besides its non-ionizing radiation source. It was done through *in-vitro* experiments of MCF-7 breast cancer cell line assay.

II. METHODOLOGY

Figure 1 below is a schematic diagram showing how the ultrasound treatment plan will be undertaken. Effects of low energy focused ultrasound waves on MCF-7 breast cancer cells will be studied and analyzed using different resonant frequencies and exposure times of modulated ultrasound waves. A cytotoxicity test will ensue to observe and measure the number of cells still alive and dead in a micro-well plate through absorbance values of the reporter molecule. Lastly, t-test will then be used to compare the metabolism of the untreated and that of the exposed cells through cytotoxicity test.

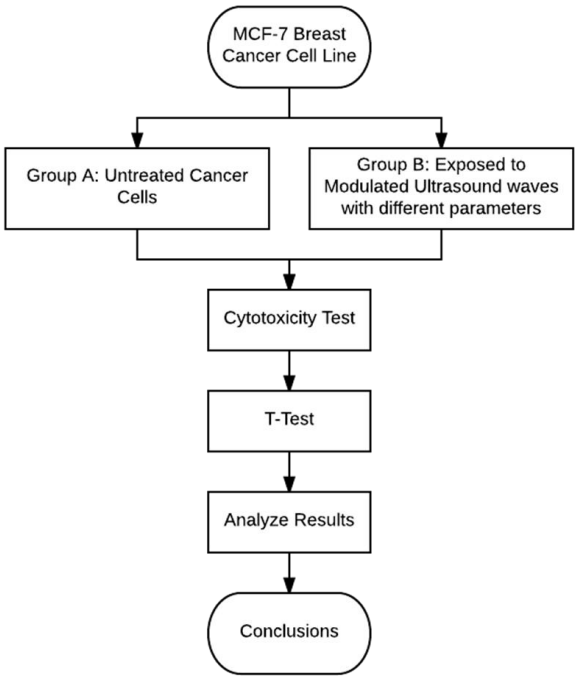

Figure 1. Schematic diagram of the experimental design

Ultrasound is known for diagnostic use in the medical field. However, recently it was discovered that ultrasound can be used for treatment as well. Ultrasound has become a common therapy for several clinical conditions. Ultrasound, other than for diagnostic purposes, has observed non-thermal and thermal effects on biological tissues. All of them can be explained through the outcomes of using ultrasonic waves on a biological substance.

All matter has vibrations and so does biological substances. An object that possesses a dimension length can be viewed as something with resonant frequency. There is a certain frequency where the vibration reaches the highest amplitude which is called resonance frequency. There are corresponding resonant frequencies for each matter which can lead to the distortion of those certain matter. The classic example is a singer shattering a glass. In this case the resonant frequency will be applied on the abnormal cells. Because of the difference in structure as between normal tissue cells and abnormal cells differ in resonant frequencies, only the abnormal cells will be affected with its corresponding resonant frequency. [6] The mechanical energy within the ultrasound wave and the shearing force of the wave combine to produce mechanical properties that perturbate the cellular membrane and the molecular structures within the cell. The central premise of the frequency resonance hypothesis is that the mechanical energy within the ultrasound wave is absorbed by proteins, altering the structural conformation of an individual protein or the function of a multimolecular complex. Moreover, the ultrasound wave may induce resonant activity in the protein, modulating the molecules or multimolecular complex's effector function [7]. With the advancement in the studies of DNA analysis, the length of a DNA and its other factors like space between bases are easily acquired [8]. In the DNA frequency theory, the wavelength is denoted by the base pairs multiplied by a constant 3.403846×10^{-10} meters (the spacing between individual bases). The speed of sound

targeting breast cancer cell line MCF-7 was collectively measured and ranging from 1577m/s to 1611 m/s, an average of 1594 m/s. Given the speed of sound and the wavelength the frequency is attainable through this equation $f = v/\lambda$, where f is the total resonant frequency, v is the velocity of sound through cell membrane, and λ is the resonant wavelength (product of the number of base pairs and base pair space constant). The frequency computed is a possible resonant frequency. To achieve a frequency along the audio range, we divide the frequency by 2 to achieve a lower "octave" (divide 2). This doubles the wavelength and acquires a low frequency possibly at audio range if divided continuously. [9]

In cooperation with the resonance theory, the resonant frequency of a DNA is crucial. The determination of the resonant frequency may contribute to the destruction of certain genes present in a cell. DNA has the capacity and information to assist in well-functioning and growth of the living cells in our body. It has been proven that DNA has dipole characteristic – meaning, directionality is present in terms of how charged molecules are in the chain. If the deoxyribonucleic acid chain were to be torn and broken down, positive charge would appear on one end, and negative to the other. This simply proves that DNA can receive and conduct elements. When two of these strands are linked, and connected properly, parallel charges would appear but polarities are different. The pairs that make up deoxyribonucleic acid are Adenine – Thymine and Guanine – Cytosine respectively. It has been proven that those negatively charged ions pass through the whole helical manner. This method determines and gathers strands that are very much sensitive to exposure in electromagnetic and ultrasound waves. As the study of radio sciences furnish, it has been known that the distance and length of an antenna would decipher how efficient it has been in responding to the wavelength energy of a transmission. Therefore, making the length of any deoxyribonucleic acid respond and resonated through the same belief [6, 7]. With this concept, it is now more capable of seeing the destruction of oncogene/ proto-oncogene that may result to breast cancer proliferation.

MCF-7 is a highly rearranged gene, meaning the chromosome suffers change in the structure and now differs from the native chromosome, briefly, a chromosomal abnormality. In this study, AMPH, CDC25A, and FOXO3 were considered. AMPH gene (Amphiphysin) is located at 7p14.1 (chromosome 7 short arm on region 1, band 4, sub-band 1). This gene is qualified under the events of rearrangements stated above. Autoantibodies against AMPH were found in a subset of patients suffering from stiff-man syndrome together with breast cancer. A rearrangement of AMPH could be the cause of the trigger for autoimmune response. CDC25A (Cell Division Cycle 25A) is a member of the CDC25 family of phosphatase. This gene is a phosphatase that removes phosphate ions out of a phosphorylated complex which in turn activates cyclin dependent kinase CDC2. DNA damage triggers the degradation of the CDC25A gene to prevent further progress of cell division with chromosomal abnormalities. CDC25A has a total base pair size of 31,513 and it is located along 3p21.31 (chromosome 3 on the short arm region 2, band 1, sub-band 31). A rearrangement to this gene may cause elevation of CDC25A which advances cell division causing proliferation of cells even when there is DNA damage, hence replicating damage DNA. FOXO3 encodes a protein called FOXO3 or FOXO3a. FOXO3 belongs to the O subclass of the forkhead family of transcription factors. FOXO3a regulates the elevation of PTEN, a phosphatase that removes a phosphate group from PIP3 and in turn inhibits the activation of Akt. The Warburg effect found out the reason to why a respiration disability of cancer cell still makes the cancer cell alive. A rearrangement to this gene can cause downregulation of the protein FOXO3a which can lead to cancer proliferation and survival. The FOXO3 gene is located at 6q21 (chromosome 6 on the

long arms region 2, band 1). This gene satisfied the condition of rearrangement number (2) as stated above [10-12].

The computed frequencies were used in the low energy ultrasound system was provided in figure 2. This was housed inside a special audio chamber in order to insulate the target cells from other ultrasound sources.

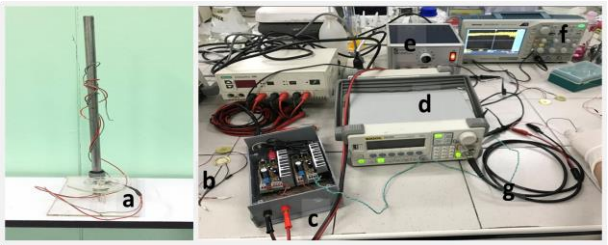

Figure 2. Low energy focused ultrasound setup. a. Three pin line b. piezoelectric transducer c. amplifier d. arbitrary waveform generator e. power supply f. digital oscilloscope g. cables.

III. RESULTS AND DISCUSSION

The effect of low energy ultrasound on breast cancer cells was analyzed by measuring absorbance values using cytotoxicity test as shown in figure 3.

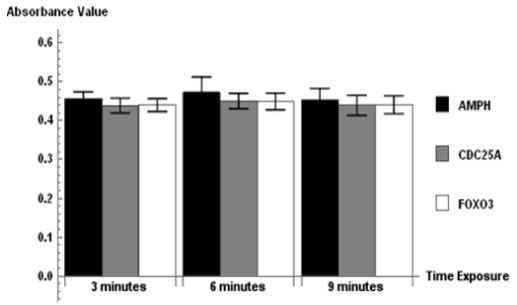

Figure 3. The Absorbance values are presented by the mean values ± standard deviation for three mutated genes of breast cancer cells lines (i.e. AMPH, CDC25A, and FOXO3) at three different exposure times (i.e. 3, 6, and 9 minutes) on 24 samples. The absorbance value for no treatment is 0.474 ± 0.012.

An independent-sample t-test was used to check the effectiveness of low energy ultrasound in the proliferation of cancer cells. Using the absorbance value under control group and the absorbance value under three mutated genes as treated groups, we were able to calculate the p-values of these treated groups with alpha level set to 0.05 (95% confidence level) as shown in figure 4.

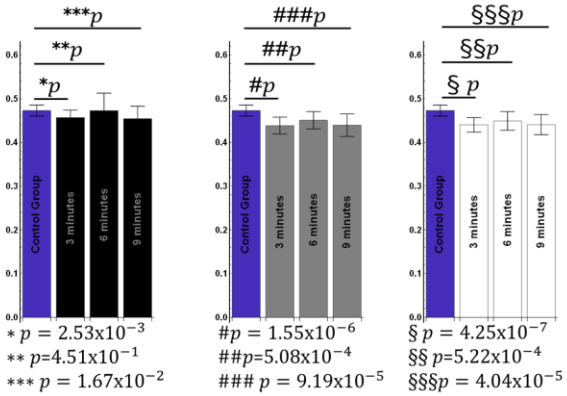

Figure 4. The Breast cancer cells are exposed to low energy ultrasound at three different frequencies (i.e. 875.71 Hz, 663.16 Hz, and 423.75 Hz) which was calculated from targeted genes of breast cancer cells (i.e. *AMPH, #CDC25A, and §FOXO3). The p values were obtained using Student's t test which shows a significant difference at 3-minute exposure. (only AMPH at 6-minute exposure time has the p value > 0.025)

T-test has shown that low energy ultrasound treatment has significant effect on MCF-7 breast cancer cells except for AMPH resonant frequency within 6-minutes which has p=9.02E-01. Statistical result has indicated that the most significant effect on MCF-7 breast cancer cell's demise is when it is exposed to 3 minutes at any of the given frequency. Also, among the resonant frequencies, FOXO3 target gene has the most significant effect compared to other resonant frequencies under study. FOXO3 has shown more consistent outcome compared to others. On the other hand, AMPH gene has shown larger absorbance values amount and larger deviation of absorbance value compared to the others, which does not show any significant effect. This might be due to frequent micro-well plate switching that might reduce MCF-7 breast cancer cell's death due to environmental exposure.

IV. CONCLSUSION

The paper presents the use of low energy focused ultrasound wave to inhibit MCF-7 breast cancer cell growth at specific resonant frequencies calculated from AMPH, FOXO3, and CDC25A target genes. Among the three resonant frequencies, FOXO3 have shown the most significant cell death of MCF-7 operating at gene's resonant frequency of 423.75 Hz. Furthermore, exposing the breast cancer cell at lower exposure time (3 minutes) would result into more significant cell deaths than other exposure times – 6-minute and 9-minute exposure times. The data obtained for different resonant frequencies indicates that low energy focused ultrasound can be an effective for cancer-specific. There are other target genes available from ATCC database that can be selected to extend this kind of study. This is just a prototype on how to calculate and induce cell death by low power ultrasound energy but it is recommended to karyotype actual biopsy samples to be able to effectively identify the chromosomal abnormalities and eventually identify specific aberrant genes for this particular mode of treatment. Since the arbitrary wave worm can generate signals up to 20 MHz, it is highly recommended to program the ultrasound wave generator to emit frequencies below 20 MHz without the use of modulation. The proposed method provides a new opportunity to be a therapeutic treatment for cancer cells.

V. ACKNOWLEDGMENTS

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