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Effect of Pulsed Electromagnetic Fields on Colon Cancer Cell Lines *(HCT 116)* Through Cytotoxicity Test

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Abstract: We studied the effects of PEMF – Pulsed Electromagnetic Field in colon cancer cell lines, in particular, *HCT 116*. Two 96-well plates were prepared to place two groups (*i.e.* control group and experimental group) of colon cancer cell lines to monitor the effects of PEMF by performing cytotoxicity test thru *Alamar Blue* dye. The frequencies used in the experiment were derived from the resonant length of the DNA base pair size of the aberrant chromosomes of *HCT 116* – chromosome *10q26*, chromosome *16p13.3*, chromosome *18p11.2*. The researchers also attempted to construct a PEMF machine with a *3.3MHz* carrier frequency and RF (radio frequency) amplifier to achieve a larger power output. The PEMF machine was tested using a digital oscilloscope and an incandescent bulb as an alternate plasma bulb. Using commercially available PEMF machine, results showed that most of the computed DNA frequencies has reduced cytotoxicity levels of the experimental group compared to the control group. This indicates that most of the cancer cells dies from the PEMF machine programmed from the calculated DNA frequencies. The *p*-value obtained from statistical *t*-test showed that most of the calculated DNA frequencies had a significant effect on the colon cancer cells since most of the values were below the alpha level (critical level) of *0.05* (95 % confidence level). Among the DNA frequencies used in the study, **656** *Hz* has shown the most significant effect in killing the colon cancer cell lines – *HCT 116*. It was shown that the we'll known 656 *Hz* DNA frequency for colon cancer cell line came from the *MC2R* gene of chromosome *18p11.2* due to its close DNA base pair resonant length proximity with calculated upper band rife frequency at 662.84 *Hz*.

Keywords: Colon Cancer; Resonance; Cytotoxicity; Electromagnetic Wave; *DNA* Base Pair Size; Medical Instrumentation

1. INTRODUCTION

Cancer is one of the deadliest diseases that ever hit the entire human species. The WHO (*World Health Organization*), conducted a statistical study last 2008 on the prevalence of cancer disease in the entire world. It shows that approximately 12.7 million cases were diagnosed with cancer while 7.6 million cases were attributed to cancer deaths [1]. The American Cancer Society has estimated that 580,350 cancer deaths and 1,620,290 new cases of cancer in 2013 year alone [2]. If this trend remained in America, how about cancer cases in other parts of the world; like in developing countries since there is a rapid increase and adaption of cancer-causing practices by economically booming countries [3]. As for the case in the Philippines, cancer is considered as the third highest ranking cause of morbidity and mortality. The leading types of cancer in the Philippines are: lung, breast, cervix, liver, colon, rectum, prostate, stomach, oral cavity, ovary and leukemia [4]. For the year 2010, the Philippine Cancer Society has projected that 82, 468 new cases and 51,808 cancer deaths [1].

Since cancer has been ranked as one of the top leading causes of death, this research will describe one alternative way on how to approach this deadly disease in particular *colon cancer*. Non-ionizing source like a PEMF operating in the mid-infrared range is used to study it's effects on *HCT116* colon cancer cell lines. Not only that, construction of PEMF can show a cost-effective way on how to develop a new treatment protocol rather than treatments used today like: chemotherapy and radiation therapy.

1.1 DNA – Frequency Theory

The DNA (deoxyribonucleic acid) is the carrier of genetic information and it has a sugar-phosphate backbone. It is composed of four nucleotides namely: Adenine which is complementary to Thymine and Guanine complementary to Cytosine. When these nucleotide

pairs are repeated they create a chain which leads to the formation of a double helix structure.

Surprisingly, the DNA has dipole characteristics, *i.e.,* there is directionality on how charged molecular components are aligned in the DNA chain. When two untwisted DNA strands are bonded and form a double helix, negatively charged ions run along the entire length on the outer surface of the chain in a helical manner. This makes the chain sensitive to the influences of electromagnetic fields *i.e.,* electric fields [5].

According to Boehm, "*I would like to interject at this point that a number ofmembrane proteins as well as DNA consist of helical coils, which may allow them to electronically function as inductor coils. Also some research that I have seen indicates that biological tissues may possess superconducting properties. If certain membrane proteins and the DNA actually function as electrical inductors they may enable the cell to transiently produce very high electrical voltages*."

In the field of radio science, the length of the antenna largely determines how effective it will response to the wavelength energy of the incoming transmission. Likewise, it has been proposed that the length of any particular piece of DNA could be resonated using the same principle [5].

Researchers continue to look for ways on how to calculate the total resonance frequency. According to the journal published by Boehm a numerical constant was found and it is 4,526,016.22 which could help in calculating the total resonance frequency [5].

Some computed frequencies could not be attained by the limitation of the generating RF source. To overcome the limitation, the frequency can be adjusted to either upper or lower harmonics from the carrier frequency. This would cut the wavelength in half or double it's wavelength. If one would want to use the lower limit, one could divide the frequency by 2 as many as necessary to reach the desired range [5].

1.2 Characteristics of HCT 116 and its associated DNA frequency

HCT116 are colorectal cancer cells derived from a human male colon having a near diploid number. The cells are epithelial in form and structure. This indicates that the cells are polygonal in shape with a regular dimension and adherent to substrates see Fig. 1.

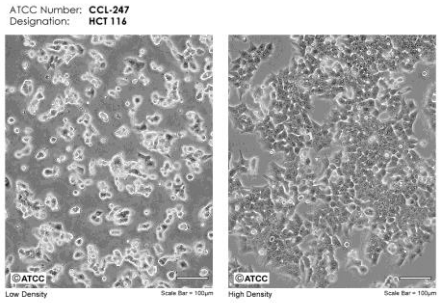


Fig 1. *HCT116* viewed under a light microscope (taken from ATCC-CCL247, 2013)

Typically, the cell line has a chromosome number of 45 occurring at 62% and polyploids at 6.8%. Karyotyping shows that 80% of the cells have an addition in the long arm (q) of chromosome 10, a translocation (t) between the short arm (p) of chromosome 8 and long arm (q) of chromosome 18 as well as 9qand 16p. While, N16 is shown to be to be unpaired (monosomic) but it is paired (disomic) if the translocation between 9q and 16p is absent. Aside from N16, N10 and N18 are monosomic and the rest of the chromosomes are disomic. The Y chromosome is also present; however, 50% of the cells do not have the Y chromosome [6].

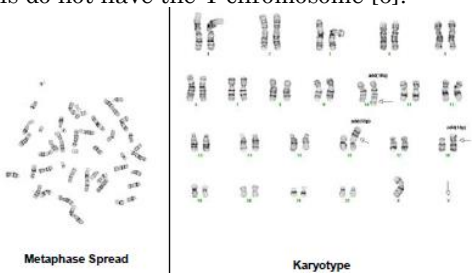


Fig. 2 Karyotype of *HCT 116* showing mutations in chromosome 10, 16 and 18.

Fig. 2 shows the mutated chromosomes namely chromosome 10, 16, 18. Chromosome 10 has an addition in it long arm specifically number 26 (*10q26*), chromosome 16 has an addition in its short arm particularly number 13.3 (16p13.3) and an addition in number 11.2 located in the short arm of chromosome 18 (*18p11.2*) [6]. Located in these chromosomal locations are the CREBBP, FGFR2 and MC2R respectively.

D.2 Chromosome 10q26

Fibroblast growth factor receptor 2 (FGFR2) gene is associated with different cancers. For certain types of stomach cancer, the *FGFR2* gene is abnormally active or overexpressed. Aside from this, abnormal expression of the *FGFR2* gene is also found in patients with prostate cancer; and an alter in the expression of

two specific FGFR2 isoforms, IIIb and IIIc, appears to correlate with prostate cancer progression. Altered *FGFR2* gene expression is also linked with ovarian, cervical, pancreatic, and head and neck cancers [7]. The FGFR2 gene location is shown in Fig. 3.

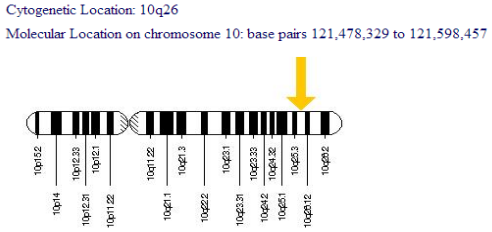


Fig. 3 FGFR2 Gene Location

D.2 Chromosome 16p13.3

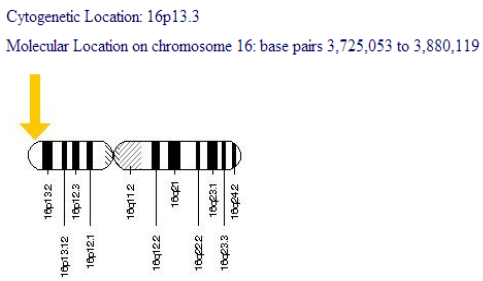


Fig. 4 CREB Binding Site

The *CREB* Binding Protein gene is associated with different types of cancer. In some cases, chromosomal rearrangements (translocations) disrupt the region of chromosome 16 that contains the *CREBBP* gene. An example of this is, found a translocation between chromosome 8 and chromosome 16 in some people with a cancer of blood-forming cells called acute myeloid leukemia (AML).Some mutations of the *CREBBP* gene was identified in several cases of ovarian cancer as well. A deletion of this protein alter the normal regulation of cell growth and division, which can allow cancerous tumors to form. [8] CREBBP location is shown in Fig. 9.

D.4 Chromosome 18p11.2

Melanocortin 2 receptor (adrenocorticotropic hormone) is a seven membrane G-Protein-coupled receptor [9]. Melanocortins are derived from the pro-opiomelanocortin. MC2R is also activated by the adrenocroticrotropic hormone and it responsible for regulating and producing steroids, circaidian vibration and the stress–related fluctuation.[10]-[11]. *MC2R* location is shown in Fig. 5.

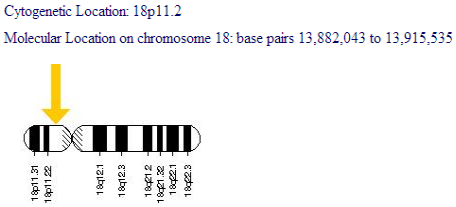


Fig 5. MC2R Gene Location

Table 1 shows the computed DNA frequency of target genes unique to *HCT 116* colon cancer cell lines. These are the resonating audio frequencies modulated by 3.3 MHz carrier frequency that will be use in the delivery the PEMF energy.

Table 1. Calculated DNA Frequency of Target Genes of *HCT 116* colon cancer cell lines

Target Gene	DNA Frequency, Hz
FGFR2 (*10q26*)	1090.29
CREBBP (*16p13.3*)	1388.00
MC2R (*18p11.2*) LB	656.00
MC2R (*18p11.2*) UB	1318.50

1.3 The PEMF

Pulsed electromagnetic field waves used by Dr. Royal Raymond Rife treated terminally ill patients for 3 minutes of exposure once every three days and it resulted into a cancer free case. This experiment was then tested by the U.S.C. Medical School Special Medical Committee during the years 1934-1935, 1937 [12].

The hunt goes on during the years as the medical field continues to find ways to eradicate cancer. Besides from the usage of radiation therapies, new technologies and procedures are rising and one of these is Bioelectromagnetics (BEMs). Bioelectromagneticsis the study of the effect of the electromagnetic field in biological systems. The benefit of BEM healing is it is able to cover a variety of illness such as cancer [13, 14].

In the medical field, PEMF therapy has been accepted and approved by some countries around the world. In the year 1979, the FDA in United States of America accepted the use of PEMF devices in healing non-union bone fractures. In 1992 it was also used for urinary inconsistence and muscle stimulation. And lastly, it was used in 2008 to cure

depression and anxiety. Israel uses PEMF device to cure migraine headaches. European Union has used PEMF in many areas including healing and recovery for trauma, degradation and treatment of pain.

The effectiveness of the PEMF therapy has been demonstrated to different kinds of painful conditions. A study was conducted where in 42 subjects who met the International Headache Society criteria were tested to see the effect of the PEMF to migraines. The results of the study showed that during the first month that there was a decrease of 75% in pain for patients who received actual treatment, 45% for substantial treatments and a decrease of 14% of excellent decrease. The researchers then concluded that for at least 3 weeks of exposure to PEMF, it is an effective and short-term intervention for migraines. Other studies were conducted to test the effectiveness of the PEMF to trauma. The study shows that 90% of patients who have dysmenorrhoea, endometriosis, ruptured ovarian cysts, acute lower urinary tract infection, post- operative hematoma, and persistent dyspareunia claimed to have relief but most of all a 10%reported that they feel less than complete pain. PEMF therapy does not only lead to the decrease of pain but it also decreases inflammation, increase cellular permeability, increase metabolism, increase cell energy storage and activity, flexibility and elasticity, and most of all it stimulates cellular and communication replication [15].

2. MATERIALS AND METHOD

The experimental design is shown below (Fig. 6) on how the effects of PEMF on *HCT 116* colon cancer lines can be quantified in terms of it's cell viability test – cytotoxicity test. Statistical *t-test* will follow to determine how significant the effect of PEMF treatments are on targeted *HCT 116* cells.

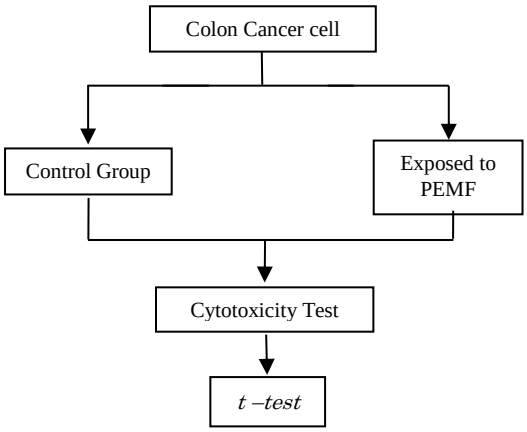


Fig. 6. Flowchart of the Experimental Design

2.1 PEMF Machine

The generation of pulsed RF wave arises from the production of audio wave generator amplified and modulated by 3.3 MHz that is appropriately tuned for a plasma tube as it's PEMF transmitting antenna. Since the energy produced by PEMF is within the mid-infrared region, the treatment wave is mostly non-absorbing to biological samples, thus the choice of RF wave is a gentle source of non-ionizing radiation delivery system for target cancer cell lines.

A laptop/Desktop is used to control the audio wave signals that would be generated. When the audio signals have been digitally programmed, the signal is then modulated by the 3.3 *MHz* carrier frequency. The modulator acts as the carrier frequency of the audio signal either in the square wave, triangular wave, or sawtooth wave to produce enough number of bands from its carrier frequency. Once the audio signal has been modulated, it is then passed through the RF amplifier. The RF amplifier increases the amplitude of the modulated signal to provide enough energy to excite the inert gas inside the plasma tube. To provide matching impedance between the RF amplifier and the plasma tube, an RF tuner is connected between the two modules. And lastly, when the specific resonant frequency has been delivered it then excites the inert gas inside the plasma tube that produces the plasma and PEMF. With two RF pulses per complex voltage oscillating cycle, PEMF are emitted. The main power for the machine to work needs to be filtered and regulates DC voltage ranging from around +100 to +200 volts [12]. Block diagram of the PEMF machine is shown below.

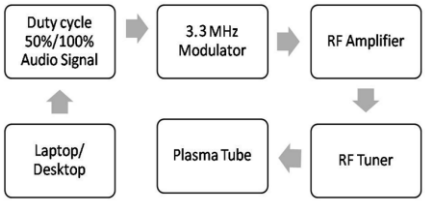


Fig. 7. Block diagram of PEMF machine

Actual PEMF is shown below with screen display that monitors the pre-programmed DNA frequencies.

(a) Screen Display (b) Plasma Tube

Fig. 8. PEMF Machine (Beam Ray) *taken from Healing Leaves Clinic*.

2.2 Colon Cancer Cell Lines (HCT 116)

The cultured colon cancer cells were provided by Dr. Sonia Jacinto from the Institute of Biology at University of the Philippines-Diliman. Then it was sub-cultured in the Molecular Biology laboratory of De La Salle University to reach at least 80% confluency. The specific type of colon cancer cell line that was used is *HCT 116*. The components of the media (see Fig. 9) used to culture the cell line are the following:

1. McCoys 5A Medium (87.5%)
2. FBS (10%)
3. $NaHCO_3$ (1.5%)
4. Pen Strep (1%)

Fig 9. *HCT 116* in culturing medium contained in a 25 mL flask.

2.3 Exposure of HCT 116 with PEMF

The PEMF machine consists of the beam ray controller and a plasma tube as shown in Fig. 10 A, B. *HCT 116* colon cancer cells are placed in each well of the 96-well plate using 5 μL micro-pipette with cell density approx. 1000 cells per well (Fig. 10 D). Each wells are covered with aluminum foil leaving the target wells open where the PEMF can be applied (see Fig. 10 C). The well plate is then placed inside the mesh containing the plasma tube (Fig. 10 A) to isolate target samples from other interfering RF signals.

A. Plasma Tube

B. Beam Ray Controller

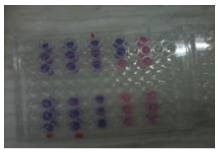
C.Cancer Cells being exposed

D. Cells not being exposed yet

Fig. 10. Set-up when *HCT 116* in a 96-well plate is exposed with PEMF.

2.4 Cytotoxicity Test

A. Alamar Blue Dye



B. HCT116 cells with the dye.

C. HCT116 cells inside the Spectrophotometer.

Cytotoxicity test was performed by adding 5uL of *Alamar Blue* fluorescent dye after PEMF treatments. The change in color of the blue dye to pink, purple or red means the cells are viable and if the color remains blue the cells are non-viable. After placing the *Alamar blue* dye on the cells, they were then placed in an incubator for 30 minutes with a temperature of 37°C. After incubation, the cells were then placed in a spectrophotometer to quantitavely determine the fluoresecnt spectral signal for each well.

3. RESULTS AND DISCUSSION

After the samples were exposed to PEMF with different resonant frequencies derived from the database sizes of the DNA base pairs of the mutated genes, the samples were categorized into a frequency sweep mode treated in 5 minutes span and a single frequency mode treated in 10 minutes span. A cytotoxicity test was followed and then we performed a statistical *t*-test to determine if there is a significant change in cell viability after the PEMF treatments.

3.1 Cytotoxicity Test Results of HCT 116 Exposed with PEMF for 5 Minutes

Table 2. Calculated *p*-Value from *t*-Test of *HCT116* Exposed with PEMF for 5 Minutes

Target Gene	Frequency Sweep, *Hz*	1	2	3	4	5	6	Ave.	*p*-value
FGFR2 (10Q26)	1521.43-2151.63	0.594	0.567	0.458	0.578	0.583	0.609	0.564	4.22×10^{-3}
CREBBP (16P13.3)	1177.90-1665.81	0.489	0.532	0.557	0.537	0.569	0.658	0.557	2.32×10^{-3}
MC2R (18p11.2) LB	1367.13-1433.42	0.510	0.545	0.528	0.501	0.499	0.660	0.541	7.01×10^{-4}
MC2R (18p11.2) UB	656	0.512	0.445	0.518	0.534	0.473	0.658	0.523	4.34×10^{-4}
Control 1	N/A	0.605	0.610	0.656	0.627	0.639	0.610	0.625	
Control 2	N/A	0.617	0.676	0.737	0.638	0.628	0.608	0.651	
Average Control		0.638							

Table 2 shows the cytotoxicity test results of *HCT 116* exposed to PEMF at different frequency sweeps for 5 minutes. Mean values greater than the average mean of the control group would indicate cell proliferation while mean values less than the average mean of the control would indicate cell death. Since all of the mean values are less than the control group, this would indicate that the cancer

cells died after PEMF treatment as indicated in Table 2.

In order to determine how significant the change was after treatment, a statistical *t*-test was performed to quanitfy whether there is a significant change in the cell's viability. Table 2 shows the calculated *p*-value of the cytotoxicity test results of *HCT 116* exposed at different frequency sweeps for 5 minutes. The *p*-value shows that there is a significant effect of the PEMF treatment with the colon cancer cell line since the *p*-values are less than the alpha level or critical level set at ***0.05***. Thus, the alternative hypothesis is accepted to be true. Results show that exposing *HCT 116* cancer cells for 5 minutes with these DNA frequencies of target genes programmed in PEMF would lead *HCT 116* colon cancer cell lines to cell death.

3.2 Cytotoxicity Test Results of HCT 116 Exposed with PEMF for 10 Minutes

Table 3. Calculated *p*-Value from *t*-Test of *HCT116* Exposed with PEMF for 10 Minutes

Target Gene	Frequency, HZ	1	2	3	4	5	6	Ave.	*p*-value
FGFR2 (10Q26)	1090.29	0.570	0.566	0.591	0.612	0.617	0.619	0.596	2.63×10^{-2}
CREBBP (16P13.3)	1388	0.787	0.598	0.451	0.559	0.545	0.549	0.582	1.28×10^{-1}
MC2R (18p11.2) LB	1318.5	0.597	0.589	0.526	0.594	0.558	0.459	0.554	1.42×10^{-3}
MC2R (18p11.2) UB	656	0.575	0.499	0.569	0.467	0.560	0.458	0.521	6.15×10^{-5}
Control 1	N/A	0.605	0.610	0.656	0.627	0.639	0.610	0.625	
Control 2	N/A	0.617	0.676	0.737	0.638	0.628	0.608	0.651	
Average Control		0.638							

Most of the *p*-values obtained are less than the alpha level of 0.05. This shows that the resonant frequencies that correspond to the calculated resonant length of the aberrant genes have resulted into significant cell death of *HCT 116*. The results show that once these genes are exposed to the resonant PEMF then it would definitely compromise the cancer cell's viability; however, for the gene CREBBP, there was no significant effect of PEFM on the cancer cell. This data might seem to contradict with the data obtained from the 5 minute frequency sweep. However, one of the frequency sweep between 1177.90 Hz and 1665.81 Hz might be the appropriate resonant frequency that could have a significant effect on *HCT 116* as shown in Table 2. But still the effect of PEFM remains the same in Table 3 but not significant to merit the result.

We have demonstrated that among the treated resonant PEMF frequencies, the resonant DNA frequency for the aberrant gene in chromosome 18 has shown the lowest *p*-value. One can deduce that the most widely used resonant frequency 656 Hz in PEMF machine might have been the resonant frequency of the aberrant gene - *MC2R* because of it's close proximity with the resonant frequency as shown in Table 3. Further pre and post test (*i.e.* karyotyping and PCA) of *HCT 116* colon cancer cell lines can be undertaken to check this possible connection.

4. CONCLUSIONS

The resonant length of chromosome *10q26* and chromosome *18p11.2* was the enabling DNA resonant frequencies that lead to the eventual death of *HCT 116* colon cancer cell lines within 10 minutes exposure time. However, the resonant length of chromosome *16p13.3* (CREBBP gene) did not have a significant PEMF effect on the viability of the *HCT 116* colon cancer cell lines. In most cases, the cytotoxicity results has shown promising and significant PEMF effect in debilitating *HCT 116* colon cancer cell lines. Among the results, the most significant PEMF effect was observed using the well-known 656 ***Hz*** DNA frequency that coincides with the initial findings of Dr. Rife. It was shown that the we'll known 656 *Hz* DNA frequency for colon cancer cell line came from the *MC2R* gene of chromosome *18p11.2* due to its close DNA base pair resonant length proximity with calculated upper band rife frequency at 662.84 *Hz*. Aside from this outcome, it was noticeable that 5 minutes exposure time is enough time for *HCT 116* colon cancer cells to die. It would be best if PEMF machine could be further be used to study it's effect on other cancer cell lines.

For other types of cancer cell lines, searching the relevant mutated genes in the aberrant chromosomes could also provide a better understanding on how PEMF can change the viability of the cancer cell lines. To assess the DNA damage of PEMF, a PCR assay test can be added to assess the extent of DNA damage done by PEMF.

5. ACKNOWLEDGMENT

The study would not be possible without the help of Dr. Sonia Jacinto of the Institute of Biology, University of the Philippines, Diliman who provided the *HCT 116* cancer cell lines for this undertaking.

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