Influence of Far Infrared Radiation on cytotoxicity of Human Breast Cancer (MCF7) cells: experimental evaluation

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Abstract. The fact that low intensity light has certain therapeutic effect has been proven through a number of research studies. There are studies that showed the applied electromagnetic radiation (EMR) in the visible and infrared light range can modulate protein and cellular activity. Here we have evaluated experimentally the hypothesis of the Resonant Recognition Model (RRM) that selectivity of protein activities is based on specific resonant electromagnetic interactions. The RRM theory proposes that an external electromagnetic field at a particular activation frequency would produce resonant effects on a protein biological activity, and this activation frequency can be determined computationally [1]. In our previous study 46 oncogene proteins were analyzed using the RRM and their characteristic frequency that correspond to their common biological activity was determined [2]. As reported in [3], the wavelengths of the applied electromagnetic radiation (EMR) in a range of 3500-6400nm are expected to affect biological activity of oncogene proteins. Thus, we designed the exposure system based on IR-LED to irradiate the selected cancer and normal cells in the wavelength range predicted computationally by the RRM [3]. The experimental evaluation of the attained far infrared wavelengths of 3400nm, 3600nm, 3800nm, 3900nm, 4100nm and 4300nm was conducted in vitro on Human Breast Cancer (MCF7) and Human Epidermal Melanocytes, which were used in this study as a control.

A comprehensive quantitative analysis of the exposed and sham-exposed MCF7 and Human Epidermal Melanocytes cells have been performed by the LDH cytotoxicity test. In addition, qualitative analysis of the effects of the applied irradiation on cancer and normal cells was performed using the light microscopy. The significance of the findings obtained from the cytotoxicity effects measured by the LDH test as well as light microscopy's results are discussed and compared with the computational predictions.

1 INTRODUCTION

According to world health organization cancer is among the top ten causes of death around the world and has higher mortality rate than traffic accidents [4]. Since cancer is the second cause of death in developed countries [5], numerous methods of treatment have been developed and attempted by scientists. One method that has proven to have effect on biological processes is low intensity light therapy which has attracted interest of cancer researchers in recent years. The concept is based on the fact that biological functions of a cell are controlled by different signals from various combinations of amino acids included in genes structure present in DNA. Any permutations in DNA of a cell which develops cancer could be hypothetically controlled if the correct signal can be exposed to the mutilated cell. Hence, exposure to the certain frequencies of irradiation should induce certain changes in functionalities of biological organism.

The main cause of cancer is uncontrolled growth due to DNA permutation. This unrestrained growth is attributed to the fact that proto-oncogenes turn their position from "off" to "on". These genes are responsible for cell division and regulation of cell death (apoptosis) and used for stimulation of cell growth and proliferation in early stages of human life. Once the process is completed, they will turn "off". However, permutation in DNA would turn them "on" and they become oncogenes, which then contribute to unregulated cell division and tumor development.

To control the behavior of oncogenes with external exposure of effective frequency signal, the revelation of correlation between its coding sequences with its biological activities is crucial. Thus, there have been several attempts for computational modeling of biological systems [6-9]. However, among them the Resonant Recognition Model (RRM) has demonstrated to be a more accurate technique for computation of frequencies, f_{RRM}, which have resonance effects on proteins biological activity [10, 11]. Since there is evidence that proteins have certain conducting or semi-conducting properties, a charge moving through the protein backbone and passing different energy stages caused by different amino acid side groups can produce sufficient conditions for a specific electromagnetic radiation or absorption. In our previous research we have shown that such charge transfer through the protein backbone is possible through an excitation process [7].

According to the RRM, a strong linear correlation exists between the predicted and experimentally determined frequencies corresponding to the absorption of electromagnetic radiation of such proteins [7], [9]. It is inferred that approximate wavelengths in real frequency space can be calculated from the RRM characteristic frequencies for each biologically related group of sequences. These calculations can be used to predict the wavelength of the light irradiation, λ, $\lambda=201/f_{RRM}$, which might affect the biological activity of exposed proteins [7], [9]. The frequency range predicted for protein interactions is from 10^{13}Hz to 10^{15}Hz. This estimated range includes IR, visible and UV light. In our previous study we used the RRM to determine the characteristic frequencies of the selected oncogene and proto-oncogene proteins [12, 13]. The results show that the frequency range of 3500nm to 6400nm is expected to have effect on the biological behavior of these proteins. Hence, it is expected that by using

the irradiation of these specific frequencies we can achieve a reduction in cell growth of cancer cells. Therefore, the exposure device was designed and developed to operate in the mentioned above frequency range [2].

There are several theoretical and experimental studies published that used visible and infrared coherent light exposures for various applications [14, 15]. However, the effect of far infrared non-coherent light exposure for cancer treatment has not been widely used so far. In this study, we investigated *in vitro* the effects of the specific far infrared frequencies (within the range of frequency determined computationally by the RRM) on Human Breast Cancer (MCF7) and normal dermal epithelial cells, which were used here as a control.

2 MATERIAL AND METHODS

2.1 MATERIALS

Lactate Dehydrogenase (LDH) Activity Assay Kit for cytotoxicity detection (Roche Australia). The culture media: clear DMEM (Invitrogen Australia). Each bottle of 500ml had 10ml of HEPES (buffer for Media) with 10% of Fetal Bovine Serum and 1% of Antibiotics (streptomycin). Trypsin-EDTA solution and Phosphate Buffered Saline (PBS) was used during the cell culture. The experiments were conducted in the incubator at the temperature of 37^{oC} with 5% CO_2.

2.2 CELL LINES

Three different patches of MCF7, Human Breast Cancer cells, with passage number of 6, 7 and 8 have been uses for triplicate of experiments. The cell lines were cultured in DMEM+10% Fetal Bovine Serum with 1% antibacterial. One patch of HEM, Normal Dermal Epithelial cells, with passage number of 3 has been used for the experiments. The cell lines were cultured in DMEM+10% Fetal Bovine Serum with 1% antibacterial.

2.3 EXPERIMENTAL PROCEDURES

In our experimental set up, we kept the intensity of the exposure constant for a better comparison factor. All the experiments were undertaken three times in triplicate for the accuracy of the results. Six frequencies were selected for the experimental evaluation of far infrared exposures: 3400nm, 3600nm, 3800nm, 3900nm, 4100nm, and 4300nm. All cell lines were seeded in the plate for 24 hrs before the start of exposures. Three types of experiments were conducted:

1.5 hrs of exposure inside the incubator followed by 0 hrs of post-exposure inside the incubator;
1.5 hrs of exposure inside the incubator followed by 24 hrs of post-exposure inside the incubator;
1.5 hrs of exposure inside the incubator followed by 24 hrs of post-exposure inside the incubator.

Then, immediately after the post-exposures, LDH Cytotoxicity test was used to detect the changes in cytotoxicity of studied cells.

Light microscopy for all 4 lenses has been used before the exposures, after the exposures and after the post-exposures. To eliminate any effects from the heat generated by the IR-LEDs used in the exposure device, we introduced a heat shield gel. The gel has shielding effect and was purchased from Inventables, USA. The gel was placed around the well from outside gaps. Before seeding, plates and with the gel inside were placed in UV camera two times for 30 min. More importantly, to eliminate any cross talk between the LEDs and the effect of two frequencies on the same well, we had empty wells around each well where the experiments were run.

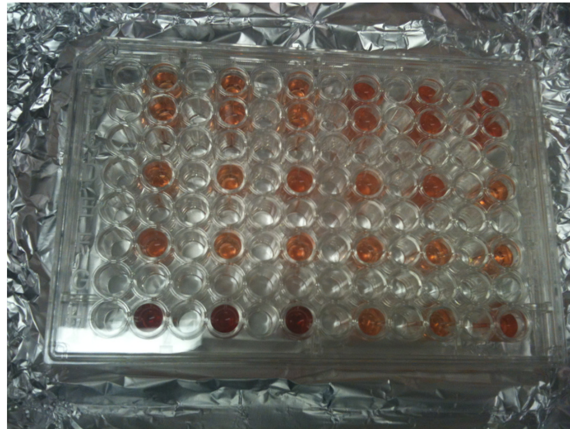

Fig. 1. A sample LDH Plate

The plates were organized as follows:

The first three permitted wells on the first row used for Medium only
The last three permitted wells on the first row used for Low Control of LDH
The first three permitted wells on the last row used for High Control of LDH
The last three permitted wells on the last row used for Low Control of LDH again.

2.4 RESULT AND DISCUSSION

The followings are the cell viability graphs from three categories of experiments that we conducted for MCF7 and HEM.

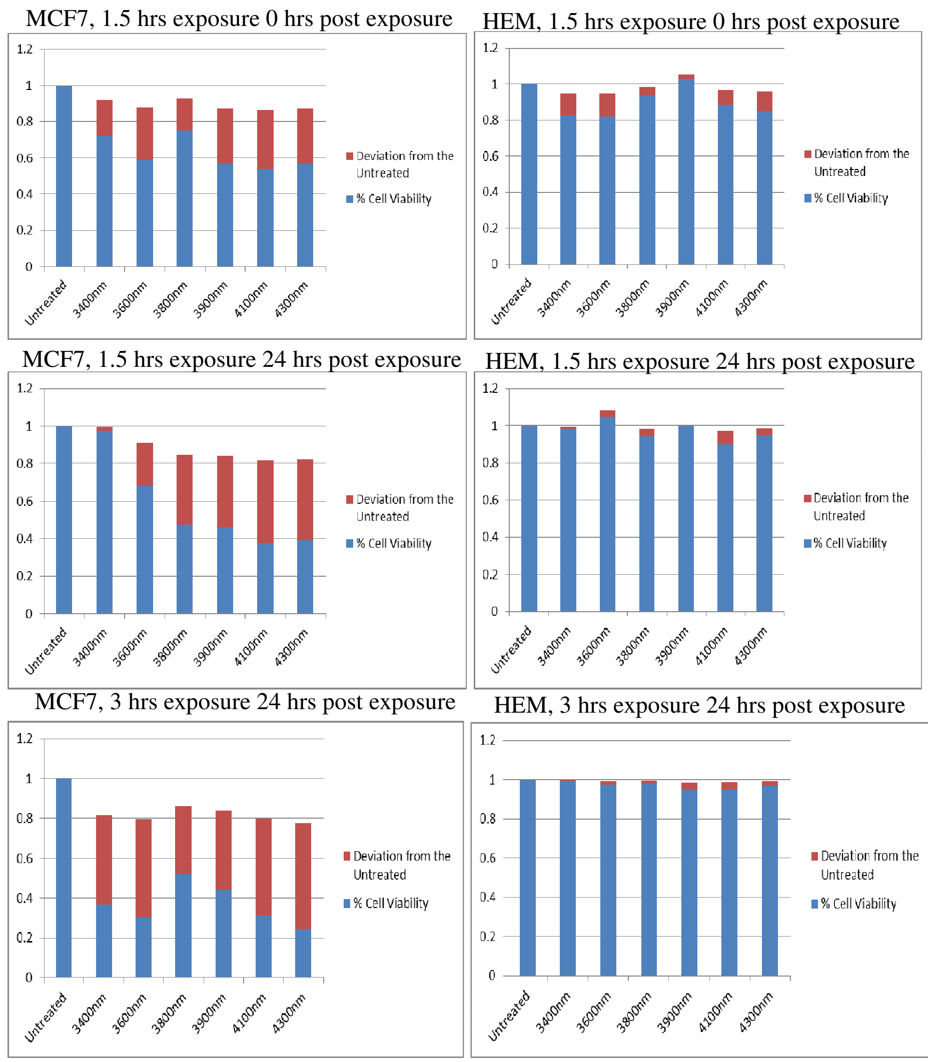

Fig. 2. Cell Viability results of MCF7 and HEM for different exposure and post-exposure times

Apparently, the cell viability of all exposed MCF7 cells at all six studied frequencies is decreased when compared to the unexposed used here as a control (Figure 2). In addition, from Figure 2 it can be seen that post-exposure has a more significant effect on the cell viability in comparison with the exposure duration. However, the irradiation of HEM cells did not produce any substantial effects on their cytotoxicity. Thus, it can be concluded that applied irradiation at the selected frequencies affected only the cytotoxicity of the cancer cells.

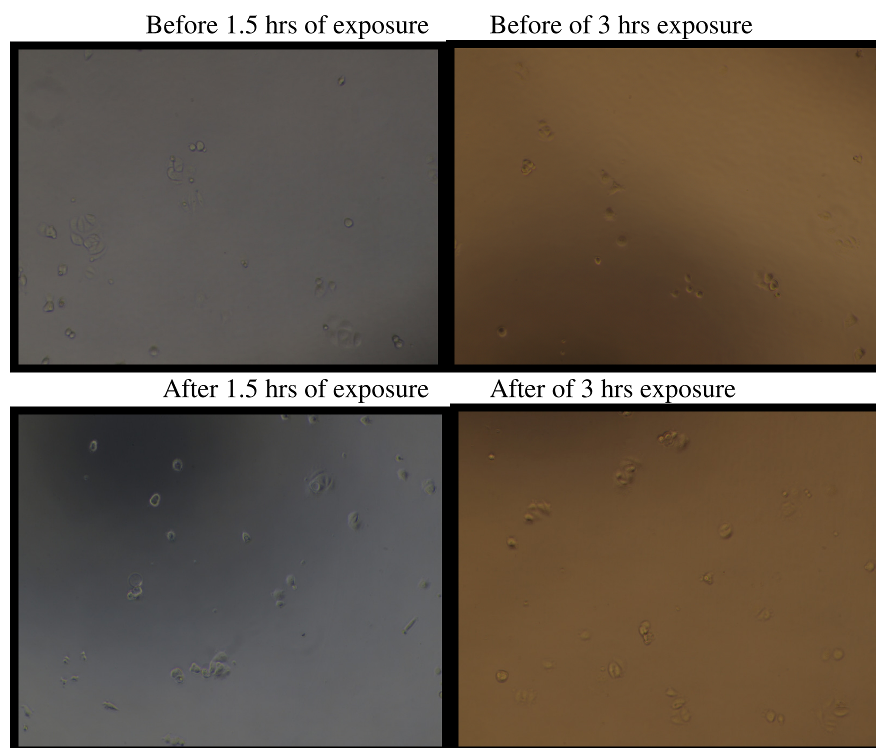

Fig. 3. Light microscopy images of MCF7, Human Breast Cancer cells, before and after exposures to far infrared light

Figure 3 shows the images obtained from the light microscopy. As we can see from the images, no observable changes in the morphology of the cells can be detected. The results indicate that there is no or little changes in the morphology of the irradiated cancer cells while LDH results clearly demonstrate a reduction in the cell viability.

3 CONCLUSIONS

In this paper we validated experimentally the hypothesis of the RRM approach that a particular biological process can be modulated by applied radiation of certain frequencies that can be defined computationally. We designed and developed the exposure system based on IR-LEDs that can emit light in the frequency range computed by the RRM. By utilizing the device we irradiated the selected cancer and normal cells by the non-coherent low intensity light at the frequencies of 3400nm, 3600nm, 3800nm, 3900nm, 4100nm, and 4300nm. The quantitative analysis of the experimental data was carried out using LDH cytotoxicity test. The results clearly demonstrated the cell viability reduction in studied MCF7, Human Breast Cancer cells while the normal HEM cells were not affected by IR-LED exposures. In addition, qualitative analysis was conduct-

ed using light microscopy. The results obtained reveal no apparent changes in the morphology of the cancer cell lines.

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