Investigation of the Effect of Extremely Low Frequency (ELF) Pulsed Electromagnetic Field (PEMF) on Collagenase Enzyme Kinetics

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Abstract: In our earlier work we have discussed the design and development of an extremely low frequency (ELF) pulsed electromagnetic field (PEMF) system that produces time varying magnetic field in the range of 0.5mt to 2.5mT. 2D and 3D simulation results of the induced magnetic field produced by the system of two pairs of air core Helmholtz coils were also presented. In this study we present the modified version of the ELF PEMF system and discuss its application to study the effect of varying parametric changes of ELF PEMF radiation on Collagenase enzymes that plays a key role in the process of wound healing. The findings from this study can be used to determine the optimal characteristics of the applied ELF PEMF for wound treatment.

1 INTRODUCTION

Power absorption in biological structures exposed to electromagnetic irradiation is a basic aspect of the study of biological effects of electromagnetic waves. Causes of induced biological effects have often been associated with the minimum electromagnetic (EM) energy necessary to affect different chemical reactions. However, electric and magnetic fields may affect the metabolically supplied energy, which might affect the rate of biologically important chemical reactions (Sebastian, 2001).

There is much evidence that experimental therapies in the vicinity of extremely low frequency (ELF) part of the electromagnetic spectrum (Juutilainen and Lang, 1997) have been emerging for various medical conditions, such as non-uniform bone fracture, skin ulcers, migraines and degenerative nerves. In clinical practice LF PEMF applications offer the possibility of more economical and effective diagnostics and non-invasive therapies for medical problems, including those considered refractory to conventional treatments. Application of PEMF to traumatic but non-infected injuries allows the tissue to repair more rapidly. Accelerating the rate of healing (Goudarzi et al., 2010) would reduce both the likelihood and effect of secondary complications.

Each and every biological process involves a number of interactions between protein and their targets. These interactions are based on the energy transfer between the interacting molecules. Enzymes are proteins crucial in accelerating metabolic reactions in the living organism. Study of biological effects of applied electromagnetic radiation (EMR) at molecular level is the main focus of this paper.

Here we aimed at investigating the efficacy of ELF PEMF to modulate the bioactivity of the Collagenase enzyme and thus to promote the rate of wound healing. We present and discuss the modified and improved ELF PEMF system which is applied to study the effects of varying parametric changes of ELF PEMF irradiation on Collagenase enzyme. We also present the findings from the experimental evaluation of the exposure system.

2 MATERIALS AND METHODS

The need of modification to our previously proposed ELF PEMF system (Ahmed et al., 2012) stems from the fact that activity of different biological samples is temperature dependent. This criterion necessitates the design for the new cuvette holder and that of the custom made temperature controlled water regulatory system.

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2.1 Improved Design of Cuvette Holder

The design of the previously used cuvette holder only sufficed for biological experiments conducted at room temperature. Conventional cuvette holders can only be used for experimental studies that require external irradiation but not in the ELF range (Pirogova et al., 2008). Utilization of commercially available cuvette holders under ELF PEMF radiation is not recommended since the metal part of the holder will interfere with the field required to irradiate the biological sample placed in the cuvette. Therefore, the need of a non-metallic cuvette holder becomes inevitable. Figure 1 shows two designs and compares between the previous and modified versions of our cuvette holder.

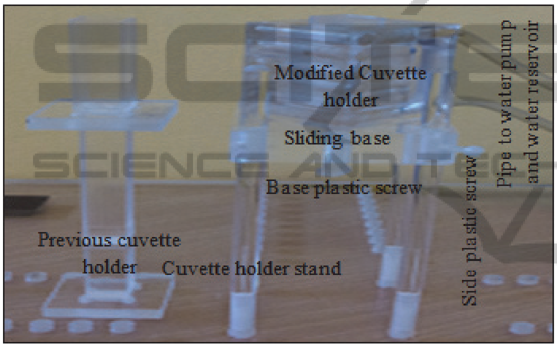

Figure 1: Visual comparison between the previous cuvette holder (left) with the modified cuvette holder (right).

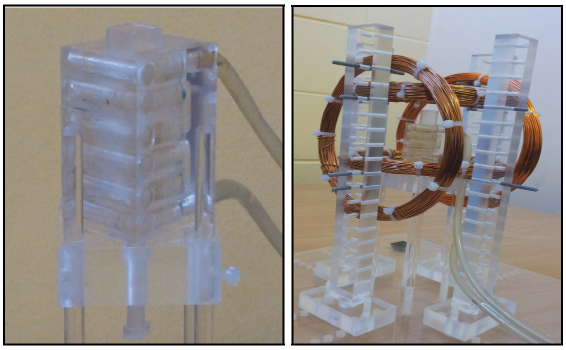

Figure 2: A close up view of the cuvette holder clearly showing the laser cut water channel (left) and the resulting modified ELF PEMF exposure chamber (right).

The entire cuvette holder setup is made of acrylic glass in order to eliminate the alteration of magnetic field produced by the system of Helmholtz coil. Four cuvette holder stands support the entire structure of the cuvette holder. The sliding base of the holder is fitted through these stands and made it stable by the slide plastic screw. This screw can easily be manoeuvred to attain the desired vertical positioning of the cuvette holder during experimentation. The base plastic screw at the bottom of the cuvette holder helps to eject the cuvette after each experiment is conducted.

Two plastic pipes are fitted to the cuvette holder (one at the top and the other above the sliding base). The pipe above the sliding base is connected to the water pump and forces temperature controlled water inside the cuvette holder through the designed laser cut water channel (Figure 2). The pipe at the top part of the cuvette holder channels the water back to the water reservoir for continuous circulation. The modified version of the ELF PEMF chamber comprising of the newly designed cuvette holder is presented in Figure 2.

2.2 Integration of Custom Made Water Regulatory System

A major challenge is to create and maintain a specific temperature in the vicinity of the cuvette. For this, a regulated water circulatory system needs to be integrated in the cuvette holder. Readily available temperature control devices cannot be used since they are connected to the holder via connectors with metallic pins. For better understanding of the working methodology of the custom made water regulatory system, a schematic design is presented in Figure 3.

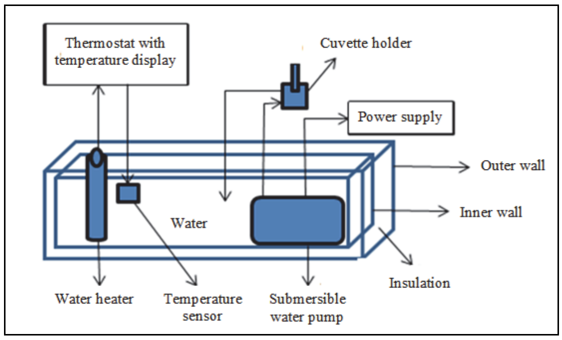

Figure 3: Schematic of the custom built water regulatory system used as part of the ELF PEMF system.

Foam board have been used as insulation material and were placed between the outer and inner walls of the water reservoir. This also ensures that the maintenance of the desired water temperature. The connection of the water pump to the cuvette holder has been presented in the section 2.1. The water reservoir also contains a water heater connected to a thermostat with a digital display of water temperatures. The temperature sensor from the thermostat is also submerged in the water reservoir.

The thermostat maintains the water temperature and accordingly turns on and off the water heater. The entire process ensures that the water temperature (in our case, 37° C) inside the reservoir is well maintained and regulated.

However, the sample temperature (with no magnetic field) also needed to be tested to see its relation to that of the reservoir's water. It was done with the help of continuous monitoring of a digital thermometer submerged into our sample of 0.1ml Collagenase enzyme solution. The time for the heat to tansfer from the water in the resrvoir to inner surface of the holder onto the cuvette and then finally to its contained sample solution was noted down. For accuracy, we repeated the process three times.

The main objective was to achieve an approximate temperature of 37° C within our sample. We observed that for our designed structure it takes approximately 56±2 minutes for the heat to transfer from the water in the reservoir to the internal area of the cuvette without the sample solution. Once the solution is put in, it takes another 2.5±0.5 minutes for the sample temperature to reach up to the desired temperature of 37° C. Once the temperature reaches 37° C (the same as that of the reservoir), the temperature remains maintained for the duration of our experiment. Therefore, before running our series of experiment and more specifically before irradiating our sample with magnetic field, we make sure that the cuvette holder is sufficiently warmed up for the heat to transfer to our solution. This is ensured by allowing our custom made water regulatory system to commence operating approximately an hour before the experiment is conducted.

2.3 Equipment

The designed ELF PEMF system was used to produce a three dimensional region of uniform magnetic field intensity in the range of 0.5-2.5mT and in the range of 2-500Hz. This field was measured by direct measurement using "Wandel and Golterman" EFA-200 EMF Analyzer fitted with an external B-probe. Also used for the experiment was Spectrometer USB2000 coupled with USB-ISS-UV/VIS (Ocean Optics, Inc.), range 190nm-870nm, CCD detector with 2048 pixels, USB-2 connection with Pentium IV (Windows XP), controlled with OOIBase32 software. Software automatically monitors and saves the absorption coefficient at 570nm every 15sec.

2.4 Measurement of Collagenase Enzyme Activity

2.5ml cuvettes are filled with the following components:

1. 25mg of Collagen Type I (SIGMA) added to 5ml of Buffer A (50mM TES Buffer with 0.36mM Calcium Chloride in 1000ml deionized water, pH 7.4) and filtered through a 0.8µm syringe filter.
2. 0.1ml of Collagenase enzyme solution (0.075 mg/ml Collagenase in Buffer A).

Experiments were performed at a temperature of 37° C. This temperature was maintained using the custom made water regulatory system described in the section 2.2. The cuvettes were filled with 0.10ml of Collagenase enzyme solution. These solutions were previously irradiated with ELF PEMF exposure system at the selected magnetic field intensities (0.5-2.5mT) for 10min.

Before considering a standard time of irradiation, we conducted our experimentation with selective frequencies over the range of 2-500Hz. We observed that irradiating our sample wih magnetic field of 0.5-2.5mT over a period of 10 minutes significantly increased the value of absorbance coefficients. However, irradiation of ELF PEMF for anything above approximately ten minutes failed to yield any significant changes in the value of absorbance coeeficeint. Therefore, the time for irradiation at all frequencies and corresponding magnetic field intensities were fixed to 10 minutes.

The irradiated enzyme solutions were then added to the already prepared solution of Collagen Type I and TES Buffer. The absorption coefficients were measured at 570nm. We repeated above experiment twice for each selected frequency (3Hz, 5Hz and 500Hz) of the applied magnetic field intensities.

3 RESULTS AND DISCUSSION

The designed and developed exposure system can be used to study the effects of ELF PEMF radiation on activity of selected protein solutions involved in the process of wound healing. In particular, results presented in this section aids to investigate the efficacy of ELF PEMF to modulate the bioactivity of the Collagenase enzyme. Experiments were conducted over the range of 2-500Hz. Selected results are presented below. Figures 4, 6 and 8 show the change in absorbance value for our irradiated and non-irradiated biological sample for 3Hz, 8Hz and 500Hz respectively. Figures 5, 7 and 9 show the relative percentage increase of the absorption coefficient corresponding to five unique values of magnetic field intensities for each frequency mentioned above. Differences were considered significant at $P \leq 0.05$.

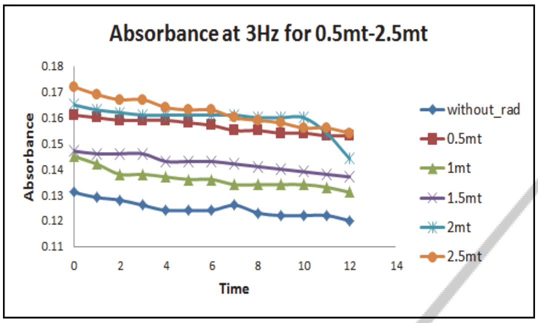

Figure 4: Changes in values of absorbance (at 570nm) in time after irradiating with 3Hz from 0.5-2.5mT.

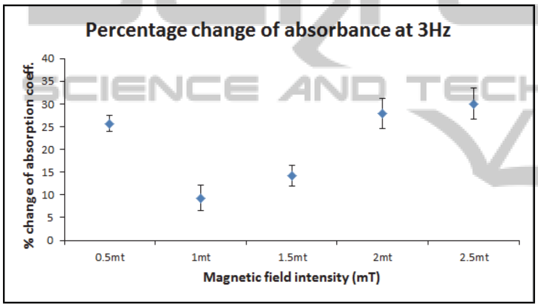

Figure 5: Magnetic field intensity dependent effect on Collagenase enzyme solution at 3Hz.

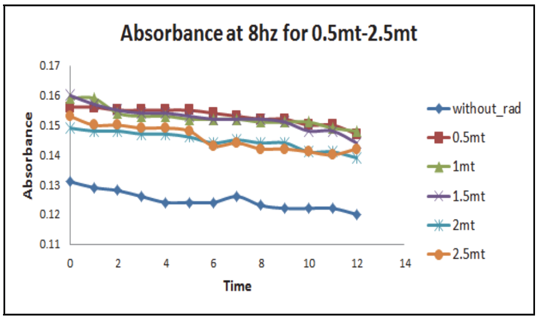

Figure 6: Changes in values of absorbance (at 570nm) in time after irradiating with 8Hz from 0.5-2.5mT.

The results showed that PEMF exposures in the frequency range of 2-500Hz and magnetic field intensity of 0.5-2.5mT increase the Collagenase bioactivity that results in accelerating the overall reaction. We can clearly observe that all values of absorption coefficient after irradiation at 3Hz and 8Hz with the magnetic field intensity range of 0.5-2.5mT yielded a positive increase of over 9.3% and 10% respectively. A significant increase of 30% was recorded at the exposures of 3Hz and 2.5mT, and of 19.1% at 6Hz and 2.5mT. The findings reveal that the overall percentage change of absorption coefficients in the range higher than 10Hz is significantly lower. The reason behind this is the fact that the percentage change in almost one or two individual values in each combination of frequency range of 12Hz-500Hz and magnetic field intensity of 0.5-2.5mt is negligible. This is clearly evident from Figure 9, where maximum percentage increase of 12.38% can be seen at 500Hz and 2mT. The minimum increase of 0.12% is observed at 500Hz and 1mT.

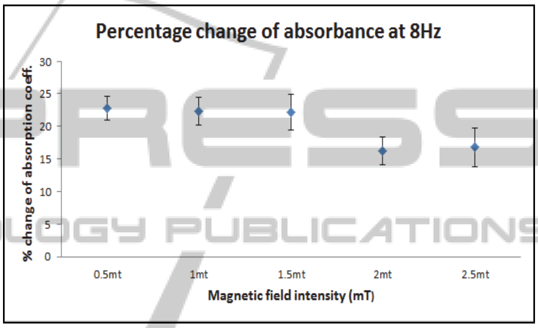

Figure 7: Magnetic field intensity dependent effect on Collagenase enzyme solution at 8Hz.

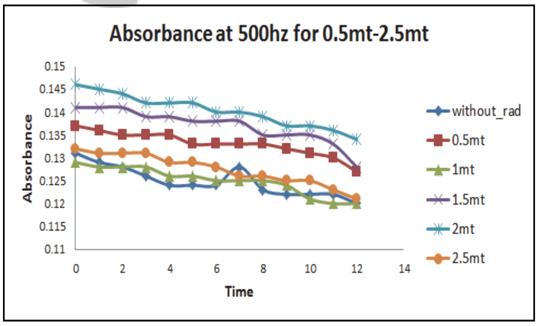

Figure 8: Changes in values of absorbance (at 570nm) in time after irradiating with 500Hz from 0.5-2.5mT.

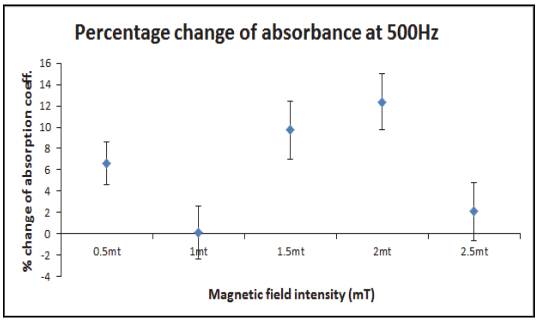

Figure 9: Magnetic field intensity dependent effect on Collagenase enzyme solution at 500Hz.

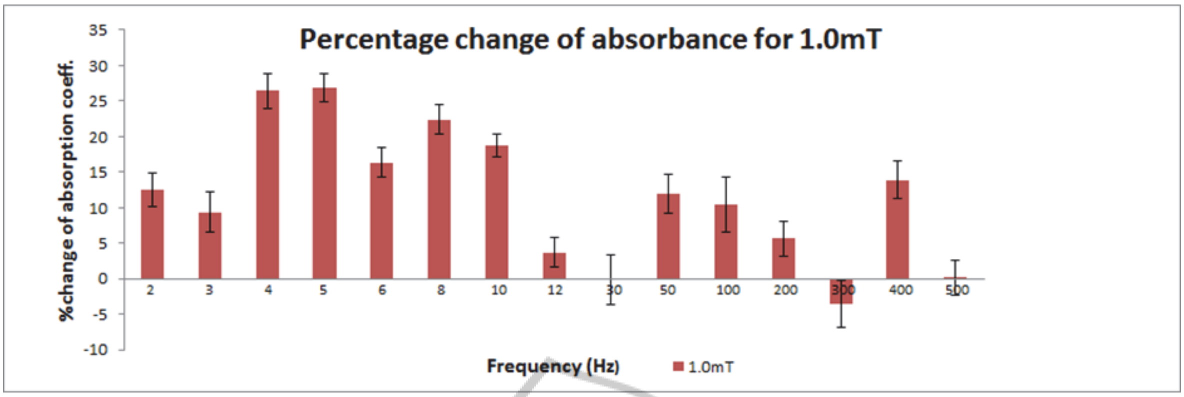

Figure 10: Magnetic field intensity (1.0mT) dependent effect on Collagenase enzyme solution for selected frequencies from 2-500Hz.

Figure 10 shows the plot of percentage change of absorption coefficient versus selected frequencies corresponding to ELF PEMF irradiation with the magnetic field intensity of 1.0mT.

Figure 10 illuminates the fact that the overall percentage increase in the absorption coefficient is indeed signficantly higer acrros the lower end of the ELF spectrum (2-10Hz) as opposed to frequencies above 10Hz. . In contrast, the percentage increase within each frequency in the range of 12-500Hz varies and is generally lower than what we find in the extremely lower end of the ELF spectrum. One possible reason might be the fact that magnetic field remains switched on for longer for lower frequencies as opposed that for higher frequencies. The relatively longer time span of the switching on and off of the magnetic field might create a significant amount of electric field inside the sample and eventually aid in the overall enhancement of Collagenase bioactivity. In summary, the results clearly show that the absorption coefficients for the entire array of 2-10Hz yield significant percentage increase.

4 CONCLUSIONS

We investigated the effects of the ELF PEMF (2-500Hz) on bioactivity of Collagenases enzyme. Prior to carrying out the experimental investigation, we made the essential modification to our previously developed ELF PEMF exposure system. The experimental results from this study clearly confirm the possibility that protein activity can be influenced/modulated by the external ELF PEMF. Findings of our investigation have direct implication in determining the optimal characteristics of the applied ELF PEMF for possible treatment of medical condition, in our case, wound healing promotion.

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