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THE EFFECT OF EXTREMELY LOW FREQUENCY (ELF) PULSED ELECTROMAGNETIC FIELD (PEMF) ON COLLAGENASE ENZYME KINETICS*

UTICAJ EKSTREMNO NISKIH FREKVENCIJA (ELF) IMPULSNOG ELEKTROMAGNETNOG POLJA NA KINETIKU ENZIMA COLLAGENASE*

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Key words
ELF PEMF, magnetic field intensity, Collagenase enzyme activity

Ključne reči
ELF PEMF, Intenzitet magnetnog polja, Aktivnost enzima kolagenaze.

Abstract

Current interest in the application of pulsed electromagnetic field (PEMF) as alternative therapy for different medical conditions has proved to be successful. A number of investigations demonstrated that low frequency PEMF enables the induction of significant current in tissues and allows for an enhanced biological effect and thus, can facilitate wound healing. In our previous study [1] we presented and discussed the design and development of an extremely low frequency (ELF) PEMF system that produces a time varying magnetic field of 0.5mT to 2.5mT the frequency range of 2-500Hz. 2-D and 3-D simulation results of the magnetic field produced by the system of two pairs of air core Helmholtz coils were also presented. In this study we present and discuss the application of the developed exposure system to study the effect of varying parametric changes of ELF PEMF exposures on Collagenase enzyme that plays a key role in wound healing. A comparative analysis of changes in the enzymatic activity of the irradiated vs. non-radiated Collagenase was performed for the selected frequencies of 2-500Hz and magnetic flux densities of 0.5-2.5mT. The results obtained reveal that the activity of a Collagenase enzyme can be modulated at the particular frequencies of ELF PEMF for magnetic flux densities between 0.5-2.5mT. The findings from this study can be used to determine the optimal characteristics of the applied ELF PEMF for wound treatment.

INTRODUCTION

Wound healing is a complex and dynamic process of restoring cellular structures and tissue layers [2]. Primary healing, delayed primary healing, healing by secondary intention and epithelization constitutes the four main categories of wound healing [2]. The wound healing process can be divided into three distinct phases: the inflammatory phase, the proliferative phase, and the remodeling phase [3]. However, certain systematic diseases such as injuries to the nervous system, metabolic changes and aging have negative impact on the healing process that leads to chronic wound formation [4]. The electro potential of healthy cells causes a steady flow of ions across the cell membrane. In an unhealthy or damaged cell, there is a sodium influx resulting in cytotoxic oedema. Injured tissues are categorized by higher healing potential as compared to unbroken skin [5].

The actual mechanism by which EMFs produce biological effects is under intense study. Evidence suggests that the cell membrane may be one of the primary locations where applied EMFs act. Electromagnetic forces at the membrane's outer surface could modify ligand-receptor interactions, which in turn would alter the state of large membrane molecules that play a role in controlling a cell's internal processes. The mechanism of action of EMF signals at the molecular and cellular level is now much better understood and strongly suggests ion/ligand binding in a regulatory cascade could be the signal transduction pathway [6,7-10]. It is reported that cells respond to the rate of change in magnetic field and not to the peak field magnitude or total flux exposure [11].

A significant number of peer-reviewed publications already showed that electromagnetic fields (EMFs) can result in physiologically beneficial in vivo [12,13] and in vitro [14,15] biological effects. Time varying EMFs consisting of rectangular or arbitrary waveforms (referred to as pulsed electromagnetic fields (PEMFs), pulse modulated radio frequency waveforms, particularly in the 15–40 MHz range (referred to as pulsed radio frequency fields (PRF), and low frequency sinusoidal waveforms (< 100 Hz) have been shown to enhance healing when used as adjunctive therapy[6]. It is known that specific changes in the field configuration and exposure pattern of low-level EMFs can produce highly specific biological responses. The high rate of change (Teslas/second) of PEMF's enables the induction of significant current in tissues and thus, allows for greater biological effects compared to that of waveforms having lower rate of change [16]. This holds true for cases where biological effects are dependent on the magnitude of induced current [16]. Detailed physics and engineering aspects of low-frequency signals were made readily available by Liboff [17].

Experimental therapies in the vicinity of the extremely low frequency (ELF 300Hz) part of the electromagnetic spectrum [18,19,20] have been emerging for various medical conditions, such as non-uniform bone fracture, skin ulcers, migraines and degenerative nerves [16]. Studies showed that the application of PEMF to damaged cells can accelerate the re-establishment of normal potentials, promote cell proliferation, increase the rate of healing, reduce swelling and bruising [16,21,22]. Future advances in wound healing will focus on affecting the agents that influence the processes of damaged tissue repair [2]. Reduced healing time can produce significant cost savings and improve patient's care [23]. A pilot study on the effect of ELF pulsating magnetic field on soft tissue injuries was carried out by Baladi et al. [24]. Several randomized clinical trials utilizing PEMF on joints and soft tissues have provided sufficient evidence on their effectiveness in accelerating healing of soft tissue injuries. Accelerating the rate of healing [21] would reduce both the likelihood and effect of secondary complications.

Up to date, research on wound healing is focused on the stimulation of cell growth, development of anti-infection treatment/agents and wound bed preparation. Wound bed preparation involves debridement and managing exudates. Enzymatic debridement may be used as the primary technique for debridement in certain cases, especially when surgical or conservative sharp wound debridement are not feasible due to a patient's bleeding disorder or other complications. Collagenase enzymes were shown to act more selectively on denatured collagen in devitalized tissue. This selectivity is beneficial as it keeps the vital tissue and growth factors crucial to wound healing intact. In our study we aim at investigating the efficacy of ELF PEMF to modulate the bioactivity of the Collagenase enzyme and thus to promote wound healing. In this study we evaluated the efficacy of ELF PEMF to modulate the enzymatic activity of the Collagenase. We developed the ELF PEMF system [1] that produces time varying magnetic fields in the frequency range of 2-500Hz and magnetic induction (magnetic flux density) of 0.5 – 2.5mT. The findings of this study are presented and discussed here.

MATERIALS AND METHODS

Equipment

1. The designed and constructed ELF PEMF system [1] (0.5-2.5mT, 2-500Hz) is shown in Figure 1.

2. EFA-200 EMF Analyzer fitted with an external B-probe (Wandel and Golterman) is shown in Figure 2.

3. 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. The software automatically monitors and saves the absorption coefficient at 570nm every 15sec.

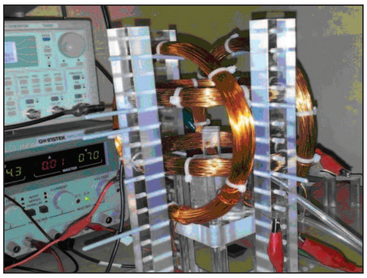

Figure1. Close view of ELF PEMF chamber

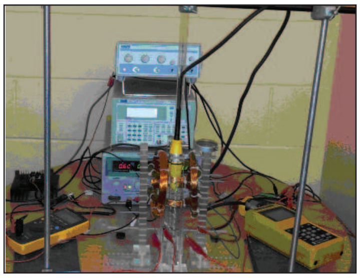

Figure2. Experimental set up for measuring magnetic flux density using EMF Analyzer

Measurement of the Collagenase Enzyme activity

The activity of the Collagenase enzyme is measured by determining the rate of substrate utilization during the enzyme-catalysed reaction. The temperature was controlled during Collagenase irradiation as well as during the activity measurement procedure. Experiments were performed at the temperature of 37C.

Irradiated sample

The cuvettes were filled with 0.10ml of Collagenase enzyme solutions. 0.1ml Collagenase enzyme solutions were irradiated by the ELF PEMF of the following characteristics: frequency range of 2-500Hz and magnetic flux density of 0.5-2.5mT. Each test consisted of irradiating the Collagenase enzyme solution for 10min at the selected values of frequency and magnetic flux density. After irradiation the Collagenase enzyme solution was added to the already prepared solution of TES buffer and Collagen Type 1. We then used the standard protocol (Sigma assay) to measure the optical density of the experimental solution at 570nm. We repeated the above experiment three times for each selected set of frequency and magnetic flux density.

Non-irradiated sample:

0.1ml of Collagenase enzyme solutions were sham-exposed and kept under the same experimental conditions for 10 min and then added to the solution of TES buffer and Collagen Type 1. Standard protocol was maintained to obtain results for the optical density of the non-irradiated sample.

The assay is presented below:

2.5ml cuvettes are filled with the following components:

● 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.

● 0.1ml of Collagenase enzyme solution (0.075 mg/ml Collagenase in Buffer A).

For every irradiated enzyme sample we have measured the absorption spectra for 10 min by recording the solution's absorption values every 15 sec as follows:

$$A_\lambda = -\log_{10}\left(\frac{S_\lambda - D_\lambda}{R_\lambda - D_\lambda}\right)$$

where S_λ is the sample intensity at wavelength λ defined as the intensity of incident light that passes through the cuvette with the sample, D_λ is the dark intensity at wavelength λ, or intensity measured on the surface of the CCD detector (spectrometer) when the light source is switched off. R is the reference intensity at the wavelength λ.The activity rate is determined by calculating the gradient of $A_{\lambda\ (t)}$.

RESULTS AND DISCUSSIONS

In this experimental evaluation we examined the effects of generated ELF PEMF's on Collagenase enzymatic activity, which is defined and calculated as the rate of change of optical density in time. Collagenase enzyme solutions were irradiated at the selected frequencies in the range of 2-500Hz and five magnetic flux densities (0.5mT, 1.0mT, 1.5mT, 2.5mT and 2.5mT). The measurements were repeated three times for each experimental setting. The data was calculated and presented in Figures 3a-3c and Figures 5a-5c.

The bars in the Figures 3a-3c represent standard deviation of the data set for the relative difference in activity between irradiated and non- irradiated samples. For each measurement, the relative difference in activity of Collagenase enzyme solutions for irradiated vs. non-irradiated samples was assessed and calculated (%) and shown in Figures 3a-3c. Figures 5a, 5b and 5c show the difference in significance between the mean values of the activities of irradiated and non-irradiated samples. This provides us with another visual representation of the changes in Collagenase activity after irradiation with different combinations of the selected frequencies and magnetic flux densities.

Of note, the rate of change of optical density per second for non-irradiated sample remains constant throughout the whole range of studied frequencies and magnetic flux densities, and hence no separate plots/tables are presented for these samples. However, the consistent average rate of change in optical density for non-irradiated sample is shown as a horizontal bold line in Figures 5a-5c. Moreover, the data for non-irradiated sample are incorporated within the relative change data for generating relevant plots.

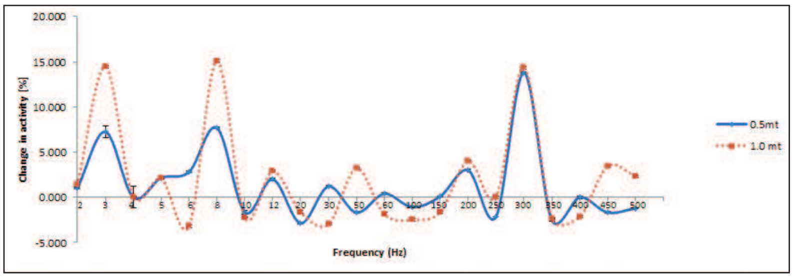

Figure 3a. Relative changes in the collagenase enzyme activity (%) induced by the ELF PEMF at the frequency range of 2-500Hz magnetic flux densities 0.5mT and 1.0mT

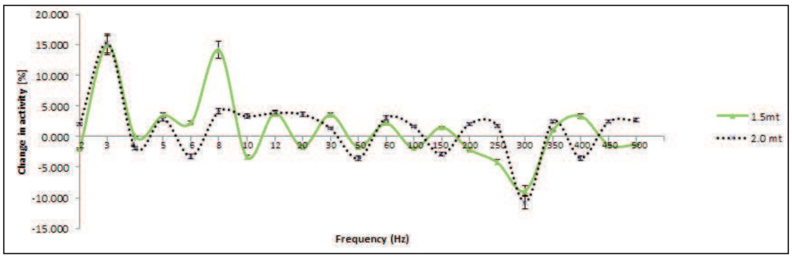

Figure 3b. Relative changes in the collagenase enzyme activity (%) induced by the ELF PEMF at the frequency range of 2-500Hz and magnetic flux densities 1.5mT and 2.0mT

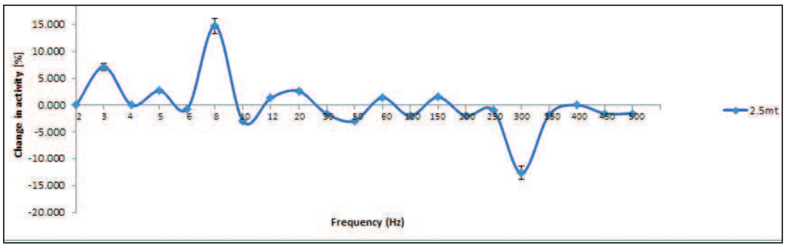

Figure 3c. Relative changes in the collagenase enzyme activity (%) induced by the ELF PEMF at the frequency range of 2-500Hz and magnetic flux density 2.5mT

The results obtained are presented in Table 1 and Figure 4a-4c. These findings reveal that the biological activity of Collagenase enzyme is increased by 7-15% and 4-15% after respective irradiation at 3Hz and 8Hz for the magnetic flux densities of 0.5-2.5mT.

It can be also seen that the biological activity of Collagenase is modulated as follows:

● Increased upon exposures at 3Hz and magnetic flux densities of 0.5-2.5mT (Figures 3a- 3c and Figure 4a).

● Increased upon radiation at 8Hz and magnetic flux densities of 0.5-2.5mT (Figures 3a-3c and Figure 4b).

● Increased upon radiation at 300Hz and magnetic flux densities 0.5mT and1.0mT (Figure 3a and Figure 4c).

● Decreased upon radiation at 300Hz and magnetic flux dencities1.5mT, 2mT and2.5mT (Figures 3b, 3c and Figure 4c).

It should be noted that for almost all studied frequencies in the lower end of the ELF spectrum (2-12Hz) we can observe an increase in Collagenase enzyme activity upon radiation with ELF PEMF. Significant changes in its activity are obtained at the frequencies 3Hz and 8Hz for all five studied magnetic flux densities (0.5mT, 1.0mT, 1.5mT,

Table 1. Activity of the enzyme and standard deviation upon irradiation with ELF PEMF

Frequency (Hz)	0.5mT activity	STD(±)	1.0 mT activity	STD(±)	1.5mT activity	STD(±)	2.0 mT activity	STD(±)	2.5mT activity	STD(±)
2	1.000	0.739	1.379	0.739	-2.000	0.167	2.015	2.429	-0.003	1.528
3	7.261	0.651	14.429	1.337	15.000	0.835	15.333	3.108	7.143	1.528
4	0.000	1.165	0.000	0.996	0.000	1.679	-1.887	0.754	0.000	1.311
5	2.130	1.165	2.071	0.996	3.500	1.679	2.889	0.754	2.698	1.311
6	2.800	0.853	-3.197	1.443	2.286	1.528	-3.234	0.793	-0.685	2.887
8	7.692	0.965	15.091	1.443	14.222	1.371	4.167	1.115	14.769	1.832
10	-1.667	1.883	-2.231	0.905	-3.351	1.000	3.333	1.954	-3.031	1.055
12	2.000	1.422	2.875	0.835	3.803	1.165	3.875	1.497	1.333	1.044
20	-2.867	1.446	-1.667	1.567	-1.636	0.953	3.665	0.953	2.615	1.240
30	1.189	0.835	-2.996	1.138	3.581	0.900	1.400	1.138	-1.590	0.522
50	-1.705	0.900	3.182	0.853	-1.680	1.658	-3.570	1.084	-2.920	0.492
60	0.380	0.900	-1.900	1.784	2.205	0.965	3.091	0.793	1.385	0.996
100	-1.091	0.900	-2.500	1.231	-1.875	1.073	1.667	0.953	-2.000	0.754
150	0.080	0.718	-1.667	0.953	1.538	1.443	-2.941	1.881	1.500	1.348
200	2.989	0.965	3.999	0.718	-2.099	0.953	2.111	0.853	-2.000	1.913
250	-2.154	1.240	0.000	1.485	-4.215	0.778	1.733	0.778	-1.000	1.206
300	13.786	0.866	14.330	1.422	-8.840	0.937	-10.768	1.279	-12.667	0.888
350	-2.500	1.371	-2.389	0.953	1.066	0.996	2.500	1.348	-1.790	1.782
400	0.000	1.403	-2.195	1.165	3.364	0.669	-3.500	1.485	0.000	1.165
450	-1.688	1.030	3.412	0.622	-1.333	1.485	2.457	0.866	-1.538	0.669
500	-1.230	0.937	2.348	0.965	-1.322	1.621	2.667	0.853	-1.578	0.900

2.0mT and 2.5mT). The non- radiated Collagenase solution has an average rate of change 0.009167 with the standard deviation ± 0.000138. The results obtained demonstrate the increase of Collagenase enzyme activity in order of 15.33% ($p < 0.001$) at 3Hz and 15.091% ($p < 0.001$) at 8Hz respectively.

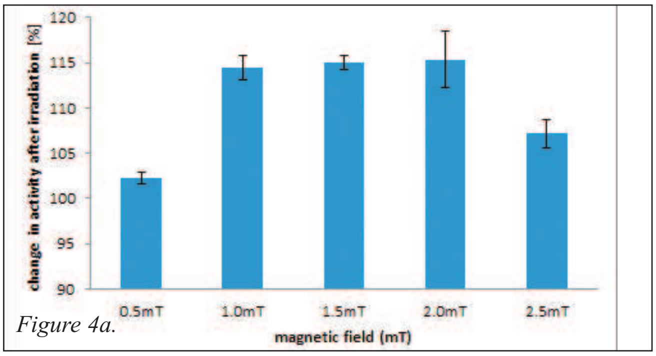

Figure 4a.

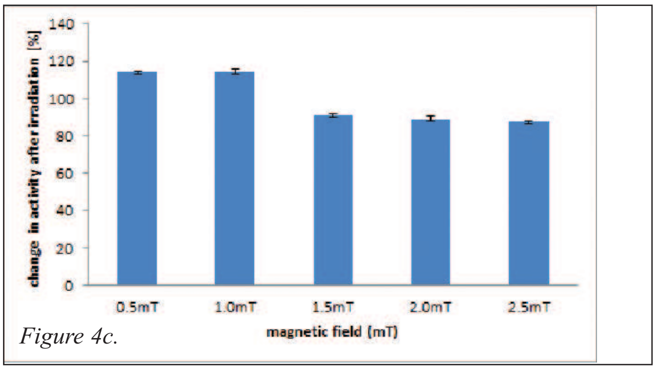

Figure 4c.

Figure 4a, 4b and 4c: Bar charts with the standard errors (standard deviation) of the relative change in the Collagenase enzyme activity (%) versus frequencies: 3Hz (Figure 4a), 8Hz (Figure 4b), 300Hz(Figure 4c) and magnetic flux dencities 0.5- 2.5mT. The percentage represents relative change to the non-irradiated samples.

Figure 5a shows the significant increase in Collagenase activity at the frequencies 5Hz, 6Hz and 12Hz and the decrease in its activity at 20Hz for the magnetic flux density 0.5mT. A significant increase in Collagenase activity can be also observed at 12Hz, 50Hz and 450Hz with a decrease in activity at 30Hz and 400Hz for the magnetic flux density 1mT. Figure 5a also shows a significant increase in Collagenase activity at 5Hz, 6Hz and 12Hz and decreased activity at 20Hz for the magnetic flux density 0.5mT. Moreover, a significant increase in Collagenase activity at 12Hz, 50Hz and 450Hz and a decrease in activity at 30Hz and 400Hz for the magnetic field intensity 1mT were

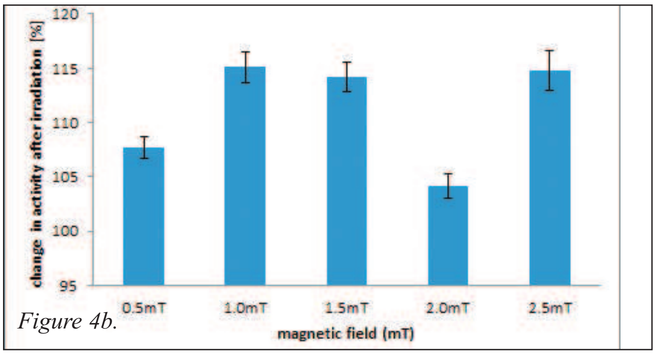

Figure 4b.

observed. Figure 5b indicates increased enzymatic activity for 1.5mT and 30Hz and 400Hz, and decreased activity at 10Hz and 300Hz. The increase in activity for 2.0mT and 10Hz, 12Hz and 20Hz, and the decrease at 6Hz are shown in Figure 5b. In Figure 5c, a notable increase in activity is observed at 5Hz and 20Hz and a decrease in activity is seen at 10Hz.

Figure 5a, 5b and 5c: The activity of the Collagenase enzyme was measured after PEMF exposures at frequencies of 2-500Hz and magnetic flux densities of 0.5-1.0mt (Figure 5a), 1.5-2.0mT (Figure 5b) and 2.5mT (Figure 5c). The ordinate represents the rate of change of the buffered solution of Collagen Type 1. The rate of change represents practically the rate of change of the buffered solution of Collagen Type I concentration per unit of time, or activity of Collagenase enzyme. Horizontal bold line represents an average (x) for the non-irradiated samples. Horizontal dash lines are values distant for one standard deviation(s) up and below for average value.

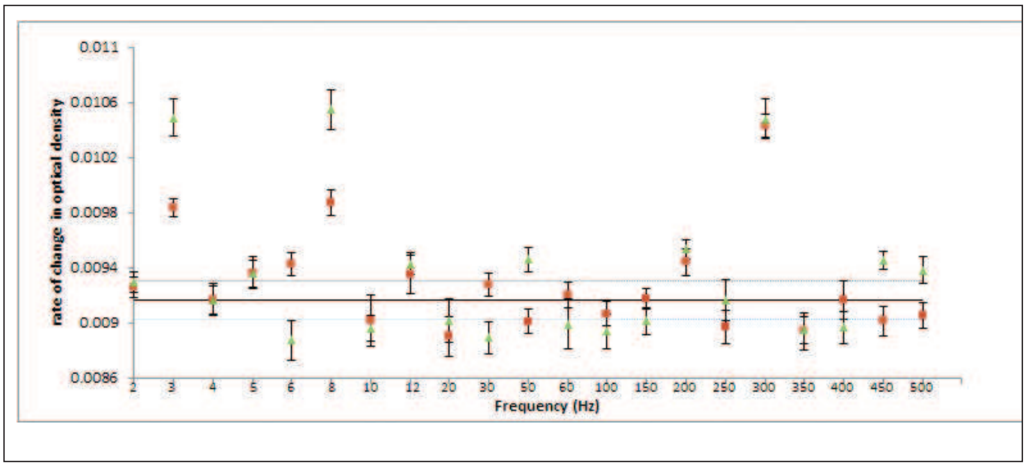

Figure 5a.

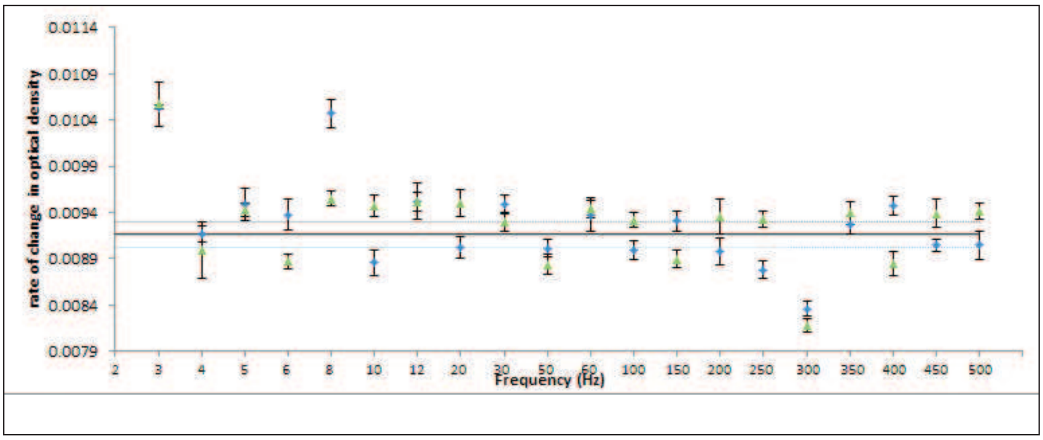

Figure 5b.

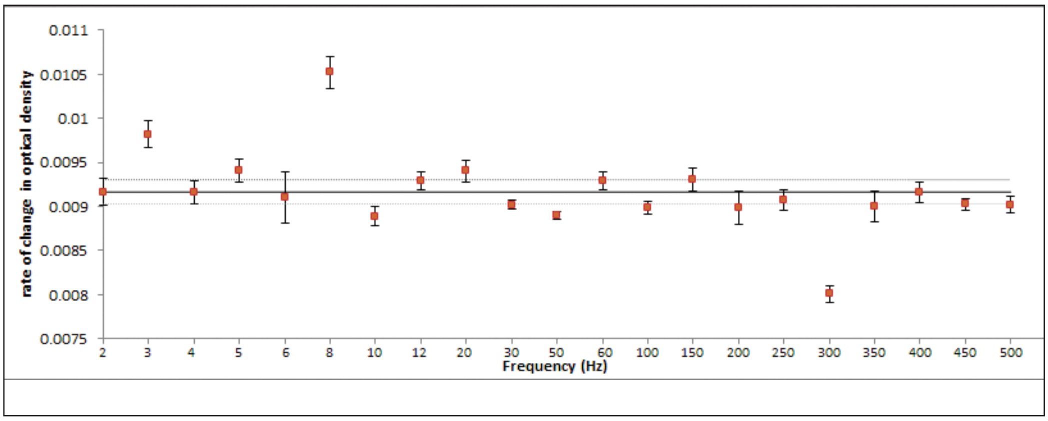

Figure 5c.

CONCULSIONS

We investigated the effects of applied ELF PEMF's on the bioactivity of Collagenase enzyme. The findings of this study show that generated fields of magnetic flux densities in the range of 0.5-2.5mT and frequencies 2-500Hz can induce changes in Collagenase enzymatic activity, and thus can affect the specific biological process involving the Collagenase enzyme. These outcomes have direct implication in determining the optimal characteristics of the applied ELF PEMF for possible treatment and promotion of wound healing.

Apstrakt

Primena impulsnog elektromagnetnog polja (PEMF) kao alternativne terapija različitih medicinskih stanja pokazala se kao uspešna. Brojna istraživanja su pokazala da niskofrekventno PEMF omogućava indukciju značajnih struja u tkivu i utiče na biološke efekte koji pomažu u lečenju rana. U našoj ranijoj studiji [1] prikazali smo i komentarisali dizajn i razvoj PEMF sistema ekstremno niskih frekvencija (ELF) koje proizvodi vremenski promenjljivo magnetno polje jačine 0.5mT do 2.5mT frekventnog opsega 2 -500 Hz. Takođe su prikazani rezultati 2D i 3 D simulacije magnetnog polja proizvedenog sistemom dva para vazdušnog jezgra Helmholcovih kalemova. U ovoj studiji prikazali smo i diskutovali primenu razvijenog sistema na ispitivanje efekata promene parametara ELF PEMF izlaganja na enzyme kolagena koji igraju ključnu ulogu u zaceljenju rana. Komparativna analiza promena u aktivnosti enzima ozračenih i neozračenih kolagena izvršena je za odabrane frekvencije u opsegu 2-500Hz a i gustine magnetskog fluksa u opsegu 0.5-2.5mT. Dobijeni rezultati potvrdjuju da se aktivnost kolagena može modulisati za određene frekvencije ELF PEMF za gustinu magnetskog fluksa u opsegu 0.5-2.5mT. Rezultati dobijeni u ovoj studiji mogu se koristiti za odredjivanje optimalnih karakteristika ELF PEMF primenjenog na tretman rana.

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