Proc. of the 5th WSEAS/IASME Int. Conf. on Electric Power Systems, High Voltages, Electric Machines, Tenerife, Spain, December 16-18, 2005 (pp219-225)

Analyzing the Action of Low Frequency Magnetic Field on Human Mesenchymal Stem Cells

Lingzhen Huang[1], Liang Dong[2], Hanshi Qi[2], Yantan Chen[2], Dengming Xiao[1*]
1 Department of Electrical Engineering, Shanghai Jiaotong University, Shanghai 200030, China;
2 School of Pharmacy, Shanghai Jiaotong University, Shanghai 200030, China

Abstract: -This paper investigates a theoretical analysis of the mechanism and experiments for the action of low frequency oscillating magnetic field on cells. The model of cells exposed to the oscillating magnetic field was described and the characteristics of ions in/out cell were studied. Time varying magnetic field and its inducing electric field produce force on the moving ions, thus accelerates the ions. On the basis of the theoretical analysis, a series of experiments were designed to test the supposed theory. Human Mesenchymal Stem Cells (hMSCs) were used in our experiment. The hMSCs were exposed to a continuous sinusoidal 50Hz, 20mT magnetic field for 23 days. The results demonstrate the significant changes of Na^+ and K^+ ion concentrations in cell supernatant comparing to the control group. These results of experiments appear to be consistent with the theoretical analysis. The results are also discussed in view of the relationship between the cell growth and ion concentration.

Key-Words: - Magnetic field, Inducing field, Displacement, Human Mesenchymal Stem Cells, Ion concentration

1 Introduction

Research groups have focused on the question whether electric and magnetic fields can affect biological systems, and how to investigate cell responses to electric and magnetic fields [1,2]. Many theoretical proposals and experimental data suggest that electric and magnetic fields act at the plasma membrane through an interaction media, which affects enzyme activities and signal transduction pathways [3,4]. Until now, the mechanism of the interaction between electric or magnetic fields and cellular systems is still unclear.

Low frequency electric field cannot penetrate the cell membrane because the lipid plasma membrane functions as an electrical insulator at low frequency [5]. But the magnetism of cell and medium can keep the magnetic field penetrating through cell membrane and extracellular medium in experiments. Thus the low frequency magnetic field can generally penetrate the cell membrane *in vitro*.

Recently, Blanchard and Blackman has used the ion parametric resonance (IPR) model [6], which is one of a series of theories proposed to explain how low frequency magnetic field might influence biological systems. Theory combining action of DC and AC magnetic fields on thermal motion of ions in a biological macromolecule is study, which explains the "frequency" and "amplitude" windows [7]. This classical model considers the Lorentz force acting on a moving charge in magnetic and electric fields. If the ions move more rapidly, the interactions with magnetic and electric fields are more effective.

On both sides of every cell membrane, there are free ions, K^+, Na^+, Ca^{2+}, etc. Ion flux through cell membrane is caused by force due to concentration and voltage gradient between the two sides of the membrane. Weak oscillating electromagnetic fields in the extremely low frequency range have been under investigation for possible effects on biological systems for some times. Panagopoulos [8] discusses a biophysical model to explain the action field on cells, analyzes forced-vibration of all the free ions on the surface of a cell's plasma membrane, caused by an external oscillating field.

Series of experiments about ions have been carried on. Suitable combinations of static and time varying magnetic fields directly interact with the Ca^{2+} channel protein in the cell membrane [9]. Exposure of HeLa cells to an intermittently switched, 1.7 T magnetic field leads to partial and significant inhibition of K^+ influx. Inhibition of K^+ influx can be due to exposure-induced direct and/or indirect inhibition of K^+ channel activity [10].

On the basis of these findings, the model includes oscillating magnetic field and the electric field induced by it was focused on in this paper. To better understand the behaviors of cells in magnetic field, a series of experiments have been carried out to confirm the theory. The human stem cells are the most effective tool to study the development and cancer occurring of human beings [11,12]. Any small influence on stem cells may cause unexpected

results to its related adult cells. Human Mesenchymal Stem Cells (hMSCs) is an important lineage in stem cell family, which can differentiate into the adipocyte, chondrocyte and osteocyte [13] in classic ways. We investigated hMSCs with respect to the effects of a 20mT 50Hz sinusoidal magnetic field on cell ion concentration. The mechanism that magnetic field inhibits cell growth *in vitro* may be related to the changes in cell ion concentration.

2 Theoretical Analysis

2.1 Cell Model

There are calcium, sodium, magnesium, potassium, hydrogen, and other ions on both sides of cell membrane. The movement of ions into the cytoplasm from the extracellular medium or through out from intracellular can be achieved through the opening of various ion permeable channels, such as Ca^{2+}, Na^{+}, K^{+} channels etc. Liboff and McLeod [14] assume that the ion in the channel is very close to the wall and is forced through the channel because of its spiral structure.

Ions play many important roles in membrane stabilization, cellular homeostasis, and process transduction in all living organisms [8,15]. The magnetic field exerts influence on the cells through interaction with moving ions.

The model of cell exposed to alternate electromagnetic field can be described as follows: for a spherical cell with a uniform membrane, the ions locate both in and out of the cell membrane, moving through the permeable channels. One ion in the cell cytoplasm was analyzed here (see Fig.1).

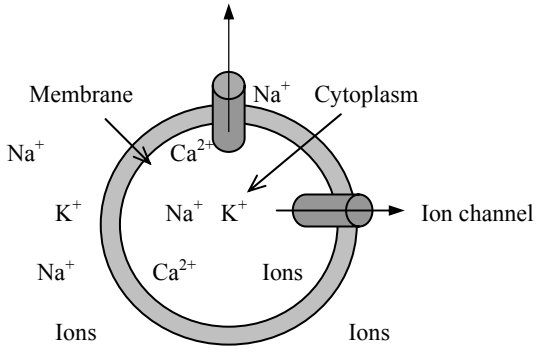

Fig.1. Model of the ions inside-out cells.

The model especially takes the ion motions into account that the ion may oscillate back and forth under the influence of magnetic field. Force generated by electromagnetic field acting on a free ion can be expressed by the Lorentz force law.

$$F = q(E + v \times B) \qquad (1)$$

Where q is the ion's electric charge, v is the ion's moving velocity, E is the electric field acting on the ion, B is the magnetic field acting on the ion. It is assumed that the ion's mass is m. In classical physics, there is no other effect of electromagnetic field. Equation (1) supposes the quantities expressed in the SI units.

With the Lorentz force law, the effects of time-varying magnetic field and its inducing electrical field are analyzed as follows.

2.2 Magnetic Field

Considering the simplest case of external, oscillating magnetic field, of intensity: $B(t) = B_0 \cos \omega t$ and the circular frequency: $\omega = 2\pi f$ (f, the frequency).

A Lorentz force: $F_B = qvB$ will act on the moving ions in the vicinity of a cell's plasma membrane. The other two forces will work at the same time: (a) A restoration force: $F_r = -Dx$, proportional to the displacement distance x. ($D = m\omega_0^2$, the restoration constant, ω_0 the ion's oscillation self circular frequency.) (b) A damping force, $F_d = -\lambda v$, where λ is the attenuation coefficient for the ion's movement. Because of the Lorentz force, restoration force and damping force, the ion will obtain acceleration a, x direction, and its movement equation will be

$$ma = -\lambda v - Dx + B_0 q v \cos \omega t \qquad (2)$$

$$m\ddot{x} + (\lambda - B_0 q \cos \omega t)\dot{x} + m\omega_0^2 x = 0 \qquad (3)$$

As we can see, $|B_0 q \cos \omega t| \le |B_0 q|$

Consider that: $\lambda - B_0 q \cos \omega t = \mathrm{H}$, H can be considered as a constant varying in a limited range.

The solution of equation (1) is

$$x = D_1 e^{\xi_1 t} + D_2 e^{\xi_2 t} \qquad (4)$$

$$\xi_{1,2} = \frac{-H \pm \sqrt{H^2 - 4m^2\omega_0^2}}{2m} \qquad (5)$$

From (5), for a typical ion, such as Na^{+}, $3.8*10^{-26}$ kg, $\lambda = 10^{-12}$ kg/s, $q \approx 10^{-19}$ coulomb, and of

the oscillations of self-frequencies range from 0.016 to 0.2 Hz [8], So $\lambda >> 2m\omega_0$.

Assumed initial conditions $x|_{t=0}=0$, $\dot{x}|_{t=0}=v_0$, it can get

$$\xi_1 \cong 0,\ \xi_2 = \frac{B_0 q\cos\omega t-\lambda}{m} \qquad (6)$$

Thus

$$x = \frac{v_0 m}{\lambda - B_0 q\cos\omega t}(1+e^{-\frac{\lambda-B_0 q\cos\omega t}{m}t}) \qquad (7)$$

Take

$$x_p = e^{-\frac{\lambda-B_0 q\cos\omega t}{m}t} \qquad (8)$$

The unit of magnetic field is a large one. It is expressed as Gauss usually. 1T＝10^4G， $\lambda >> q$, so $\lambda >> B_0 q$ 。

From equation (8) it can be seen that x_p drops to zero quickly with time increasing when t=0, x_p = 1.

The relationship between ωt and the amplitude of displacement x can be expressed as below:

$$x = \begin{cases} 2\times\dfrac{v_0 m}{\lambda - B_0 q} & \omega t = 0 \\ \dfrac{v_0 m}{\lambda + B_0 q} & \omega t = n\pi, n = 1,3,5... \\ \dfrac{v_0 m}{\lambda - B_0 q} & \omega t = n\pi, n = 2,4,6... \end{cases} \qquad (9)$$

2.3 Inducing Electric Field

The relative magnetic permeability of biological tissues is about 1 [8]. Therefore, the magnetic field's intensity within the cells will be almost equal to the intensity outside.

When a time-varying magnetic field B is exerted on the cell, it induces the electric field E interior cell. According to Faraday's Law of Induction,

$$\oint \vec{E}\cdot\vec{dl} = -\frac{d}{dt}\iint \vec{B}\cdot\vec{dS} \qquad (10)$$

The ion's distance from the center of the cell is r . Taking a typical effective area as that of a circle with a radius of r, the induced electric field

$$E(t) = -\frac{r}{2}\frac{dB(t)}{dt} = \frac{r}{2}B_0\omega\sin\omega t \qquad (11)$$

The movement equation will be

$$\oint \vec{E}\cdot\vec{dl} = -\frac{d}{dt}\iint \vec{B}\cdot\vec{dS} \qquad (12)$$

The solution will be

$$x = \frac{rqB_0}{2\lambda}(\cos\omega t-1)+\frac{v_0 m}{\lambda}(1-e^{-\frac{\lambda}{m}t}) \qquad (13)$$

From equation (13), it can be derived that the relationship between ωt and the amplitude of displacement x is

$$x = \begin{cases} 0 & \omega t = 0 \\ -\dfrac{rB_0 q}{\lambda}+\dfrac{v_0 m}{\lambda} & \omega t = n\pi, n = 1,3,5... \\ -\dfrac{rB_0 q}{2\lambda}+\dfrac{v_0 m}{\lambda} & \omega t = n\pi, n = 2,4,6... \end{cases} \qquad (14)$$

3 Experimental Procedure

Exposure of biological cells to magnetic fields can lead to a variety of profound biochemical and biophysical effects. To better understand the behaviors of cells in magnetic field, series of experiments were carried out to prove the effects of magnetic field on cells.

3.1 Materials and Methods

3.11 MSCs Culture

Human Mesenchymal Stem Cells (hMSCs), derived from thighbone and tibia of 12-week-old spontaneously aborted fetus, is a gift from Prof. Li [16], Peking University Stem Cell Research Center, China.

The hMSCs were cultivated in the Minimum Essential Medium Alpha Medium (α-MEM; GIBCO, USA) containing 10% (v/v) fetal bovine serum (GIBCO), 2.2g/L NaHCO3 (Sigma, USA) and 2mM L-glutamine (GIBCO). The cells were grown on the 96-well plate (Costar®, USA) and maintained at 37ºC in a humidified 5% CO_2 incubator (HERAcell 150, Germany). For subculturing and cell collection, the hMSCs were first rinsed twice with phosphate-buffered saline (PBS), and then trypsinized with Trypsin-EDTA (0.05% Trypsin/0.53 mM EDTA). The cells were all inoculated at the density of 2.0×10^4 cells/mL and media were changed at day 3. From then on, the media were changed every 4 days.

3.12 Electromagnetic Field Exposure System

A continuous sinusoidal 50Hz field generated by a solenoid coils exposure system was employed in the experiments of this article. The system's designer was Department of Electric Engineering (Shanghai Jiao tong Univ. China). Solenoid coils were located inside incubator horizontally. The generated magnetic field was vertical. The exposed groups were placed on a Plexiglas slab in the center of the solenoid coils, where the magnetic field is most intense and uniform. The solenoid was connected to a step-down transformer and to a variable transformer with an affixed scale that was plugged in to a 50Hz, 220V AC source. The current flowing into coils is observed by a multimeter. When the coils are energized, the 50 Hz sinusoidal magnetic fields can be regulated from 0 to 30mT.

3.13 **Exposure protocol**

The hMSCs were inoculated to the 96-well plate (100μL/well) at the density of 2.0×10^4 cells/mL. After 12 hours, the exposure groups were exposed to the 50Hz, 20mT magnetic field for 12 hours, and since then on they were exposed 12 hours/day during the whole culture period. Control groups were cultured in another incubator, in which the condition was the same as the exposure groups but free of 50Hz, 20mT magnetic field.

3.14 **Extracellular Ion Concentrations**

Supernatants were removed for assay from 96-well plate before changing media. Na^+ and K^+ ion concentrations in the culture supernatants were measured using a Nova -BioProfile 100 Plus Biochemistry analyzer (Nova Biomedical, USA).

3.2 Results

3.21 Extracellular Na^+ and K^+ Ion Concentrations

Free Na^+ and K^+ ion concentrations are strictly regulated inside and outside the cell. The intracellular K^+ concentration is of twenty to thirty orders of magnitude higher than those found in the extracellular space, and the intracellular Na^+ concentration is of ten orders of magnitude lower than the extracellular one. The influence of magnetic field on the free Na^+ and K^+ ion concentrations in supernatant was studied. Ion concentration was determined at fixed time (1, 2, 3, 4 day).

Fig.2 and Fig.3 report the Na^+ and K^+ ion concentrations in the culture supernatant as a function of time up to 23 days for both exposed and control hMSCs. The data presented above indicate that exposure to magnetic field at a limited range of flux densities may induce changes in the extracellular Na^+ and K^+ ion concentrations. The Na^+ and K^+ ion concentrations in the changed media were 138.3 mmol/L and 5.79 mmol/L, respectively. With the culture time increasing, the Na^+ ion concentrations increased correspondingly before day 15. At day 15, the Na^+ ion concentration reached the peak at 158.4 mmol/L and 161.6 mmol/L for control and exposed groups and then declined. Comparing the exposed group and control group, the Na^+ ion concentration of exposed one was higher than that of the control one at the same measured day. The trend of K^+ ion concentration was similar to Na^+ ion concentration. The K^+ ion concentration reached the peak at 6.73 mmol/L and 6.88 mmol/L for control and exposure group after 15 days culture. But at day 7, the K^+ ion concentration of control group was higher than that of exposure one.

In the other word, time varying magnetic field directly interacted with cells, and activated the efflux of Na^+, K^+ ions from the Na^+, K^+ channels, which resulted in increased extracellular Na^+, K^+ ion concentrations.

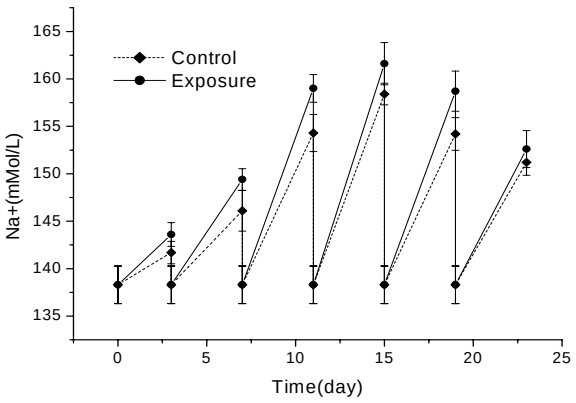

Fig.2. Effects of 20mT, 50Hz magnetic field on the Na^+ concentration in supernatant of hMSCs. All data points are plotted as mean ± SD (n = 6).

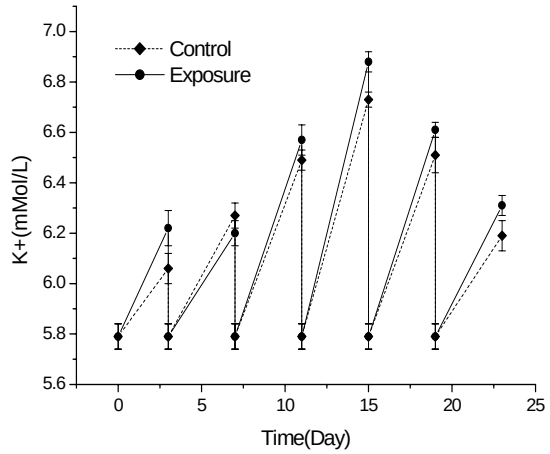

Fig.3. Effects of 20mT, 50Hz magnetic field on the K^+ ion concentrations in supernatant of hMSCs. All data points are plotted as mean ± SD (n =6)

4 Disccusion and Conclusion

Until now, there is not any generally accepted mechanism to explain the action of weak electromagnetic on cells [17,18]. In theoretical analysis, the displacement of low frequency magnetic field on the ions of cells was calculated. The mass, charge of ions, the intensity of magnetic field and the initial velocity of ions determine the amplitude of displacement. It can be derived that the inducing electric field by magnetic field also accelerates the ions through electric field force. When the amplitude of the ions vibration exceeds some critical value, the oscillating ions can give a false signal for gating channels, disorder the electrochemical balance of the plasma membrane and therefore the whole cell function. It suggests that the amplitude of the forced-vibration does not change with the magnetic field frequency, which is found in electric field [8].

Our group demonstrated that magnetic field could inhibit the growth of hMSCs recently. The results were presented at the 8th Annual Tissue Engineering Society International (TESI) Conference & Exposition [19]. With the same protocols described in our conference paper, the hMSCs in control group showed significantly more rapid growth than the corresponding exposed cells. Exposure and control groups reached their maximum cell numbers at day 17 simultaneously, while the cell number of control group was 29.25% higher than that of the exposure group, and then declined.

In this study we utilized Human Mesenchymal Stem Cells (hMSCs) with the aim of investigating the effect of low frequency magnetic field on stem cells. Our results showed that magnetic field had pronounced effects on cell ion concentration and osmolarity. As mentioned above, 50Hz, 20mT magnetic field resulted in proliferation inhibition of hMSCs. Decreased proliferation was paralleled by a significant increase of the extracellular ion concentration and osmolarity. The Na^+, K^+ ion concentrations in supernatant varied significantly between control and exposed groups after the exposure of 50Hz, 20mT magnetic field, as shown in Fig.2-3. At the same time, exposure of magnetic field increased the osmolarity. In summary, the decrease of proliferation corresponded to the increase of ion concentration and osmolarity.

Because the cell membrane interacts with environment, it will be especially vulnerable to external stimuli such as magnetic field, which may then lead to changes of crucial cellular functions. Any change in membrane transport activity detected by the movement of molecules and ions across the plasma membrane, may consequently lead to alterations in metabolic activity [20]. Blank and Soo [21] have shown that exposure to an electromagnetic field accelerates Na, K-ATPase Reactions

The effects here may be due to the interaction between time varying magnetic field and its inducing electric field and intracellular ions. The fields produce forces on the moving ions. Active ions can be bound to a channel protein and influence the opening state of the channel. These changes may relate to the permeability of the cell membrane. The movement of Na^+, K^+ into medium from intracellular cytosol can be achieved through the opening of various Na^+, K^+ permeable channels; thereby it increases the extracellular ion concentration. Transformations on molecular level can result in apoptosis [22]. It is possible that cell growth inhibition *in vitro* relates to the changes in cell ion concentration under magnetic field exposure.

In conclusion, our in vitro study suggested that the 20mT, 50Hz magnetic field used in the present investigation changed the cell proliferation and ion concentration of hMSCs. The results of experiments appear to be consistent with the theoretical analysis. More investigations may help us understanding the possible interaction mechanisms of cells and magnetic fields.

Acknowledgment

This work was supported through the grant of National Natural Science Foundation of China (No. 50477007). Authors wish to thank prof. Li Lingsong

for offering us hMSCs, and Luo Lin (Hua Yue Enterprise Holdings Ltd.) for technical support, and Songlin Qin (Department of Electrical Engineering) for equipment designing.

References:

[1] M. Blank and R. Goodman, Comment: A Biological Guide for Electromagnetic Safety, The Stress Response, *Bioelectromagnetics,* Vol.25, 2004, pp. 642-646.

[2] V. Vukicevic and K. Kampfinger, Influence of altered apoptosis in human lymphoblastoid cell lines on micronucleus frequency, *Toxicology Letters,* Vol.147, N.2, 2004, pp. 187-195

[3] F. Gobba and D. Malagoli, Effects of 50 Hz Magnetic Fields on fMLP-Induced Shape Changes in Invertebrate Immunocytes:The Role of Calcium Ion Channels , *Bioelectromagnetics,* Vol.24, 2003, pp. 277-282.

[4] C.L.M. Baureus Koch, Interaction Between Weak Low Frequency Magnetic Fields and Cell Membranes, *Bioelectromagnetics*. Vol.24, 2003, pp,395–402.

[5] C.G. Yao and C.X. Sun, Experimental Studies on Killing and Inhibiting Effects of Steep Pulsed Electric Field (SPEF) to Target Cancer Cell and Solid Tumor, *IEEE TRANSACTIONS ON PLASMA SCIENCE,* Vol.32, N.4, AUGUST 2004, pp. 1626-1633.

[6] JP. Blanchard and CF. Blackman, Clarification and application of an ion parametric resonance model for magnetic field interactions with biological systems, *Bioelectromagnetics,* Vol.15, 1994, pp. 217-238.

[7] M. Zhadin and F. Barnes, Frequency and Amplitude Windows in the Combined Action of DC and Low Frequency AC Magnetic Fields on Ion Thermal Motionin a Macromolecule: Theoretical Analysis, *Bioelectromagnetics,* Vol.26, 2005, pp. 323-330.

[8] D.J. Panagopoulos, and A. Karabarbounis, Mechanism for action of electromagnetic fields on cells. *Biochemical And Biophysical Research Communications,* Vol.298, N.1, 2002, pp. 95-102.

[9] C.L.M.B. Koch and M. Sommarin, Interaction Between Weak Low Frequency Magnetic Fields and Cell Membrane,. *Bioelectromagnetics,* Vol.24, 2003, pp. 395- 402.

[10] I. Toshitaka and K.H. Park, Effects on $Rb^{+}(K^{+})$ Uptakeof HeLa Cells in a High K^{+} Medium of Exposure to a Switched 1.7 Tesla Magnetic Field, *Bioelectromagnetics,* Vol.21, 2000, p p. 228-237.

[11] T. Reya and S.J. Morrison, Stem cells, cancer, and cancer stem cells, *Nature*, Vol.414, 2001, pp. 105-111.

[12] K.D. Amour and F.H. Gage, New tools for human developmental biology, *Nature Biotechnology,* vol.18, 2000, pp. 381-382.

[13] M.F. Pittenger and A.M. Mackay, Multilineage Potential of Adult Human Mesenchymal Stem Cells, *Science,* Vol.284, 1999, pp. 143-147.

[14] AR. Liboff and BR. McLeod, Kinetics of channelized membrane ions in magnetic fields, *Bioelectromagnetics,* Vol.9, 1988, pp.39–51.

[15] RK. Adair, A physical analysis of the ion parametric resonance model, *Bioelectromagnetics,* Vol.19, 1998, pp.181-191.

[16] X. Bai and Z. Xiao, Cartilage-derived morphogenetic protein-1 promotes the differentiation of mesenchymal stem cells into chondrocytes, *Biochemical and Biophysical Research Communications,* Vol.325, 2004, pp. 453-460.

[17] F.I. Wolf and A. Torselloa,, 50-Hz extremely low frequency electromagnetic fields enhance cell proliferation and DNA damage: Possible involvement of a redox mechanism, *Biochimica et Biophysica Acta,* Vol.1743, 2005, pp. 120-129.

[18] J. Naarala and A. Hoyto, Cellular effects of electromagnetic field, *Atla-Alternatives To Laboratory Animals,* Vol. 32, N.4, 2004, pp. 355-360.

[19] L. Dong, Y. Chen, L. Huang, etc. Effects of Extremely Low Frequency Magnetic Field on Growth, Metabolism and Differentiation of Human Mesenchymal Stem Cells. *8th Annual Tissue Engineering Society International (TESI) Conference & Exposition,* Abstract 588, Shanghai,P.R. China, Oct 22-25, 2005

[20] B.C. Stange and R.E. Rowland, ELF Magnetic Fields Increase Amino Acid Uptake Into Vicia fabaL.Roots and Alter Ion Movement Across the Plasma Membrane, *Bioelectromagnetics,* Vol.23, 2002, pp. 347-354.

[21] M. Blank and L. Soo, Optimal Frequencies in Magnetic Field Acceleration of Cytochrome Oxidase and Na, K-ATPase Reactions, *Bioelectrochemistry,* Vol.53, 2001, pp. 171-174.

[22] K. Narita and K. Hanakawa, Induction of apoptotic cell death in human leukemic cell line, HL-60, by extremely low frequency electric magnetic fields: Analysis of the possible mechanisms in vitro, *In Vivo,* Vol.11, 1997, pp. 329- 336.

[23] F. Martínez. and J.P. Pardo, The Effect of Osmolarity on Human Placental Mitochondria Functio,. *The International Journal of*

Biochemistry and Cell Biology, Vol.27, N.8, August, 1995, pp. 795-803.