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Portable device for magnetic stimulation: Assessment survival and proliferation in human lymphocytes

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A device's instrumentation for magnetic stimulation on human lymphocytes is presented. This is a new procedure to stimulate growing cells with ferrofluid in vortices of magnetic field. The stimulation of magnetic vortices was provided at five different frequencies, from 100 to 2500 Hz and intensities from 1.13 to 4.13 mT. To improve the stimulation effects, a paramagnetic ferrofluid was added on the cell culture medium. The results suggest that the frequency changes and the magnetic field variation produce an important increase in the number of proliferating cells as well as in the cellular viability. This new magnetic stimulation modality could trigger an intracellular mechanism to induce cell proliferation and cellular survival only on mitogen stimulated cells. © *2013 AIP Publishing LLC*. [http://dx.doi.org/10.1063/1.4819796]

I. INTRODUCTION

The magnetic stimulation is a technique that has been used in different study areas, such as nuclear magnetic resonance and biomagnetic signals in magneto-encephalography, thus like the effect of magnetic field on living organisms for fermentation process and some biological self-organization studies.[1–8]

Biological side effects in systems exposed to a magnetic field have been studied in the last decades. Many studies were published about the magnetic stimulation on the algal growth and its nutrition composition, the ability of prokaryotic microorganisms to activate strategies in adapting themselves to the environmental stress induced by exposure to extremely low frequency electromagnetic fields, the growth of yeasts *Saccharomyces cerevisae*, the magnetic field effect in children with acute lymphoblastic leukemia, among other.[9–15]

The implementation of a portable magnetic stimulator device for growing cells presented in this work was tested in a human lymphocytes pilot study from healthy volunteer donors. The effect of magnetic field exposition was analyzed by flow cytometry (proliferation and cell survival), which one is an instrument largely used in biomedical applications to determine cell division, clinical pathology in immunology, analysis of bacteria, and mammalian sperm in the areas of reproductive toxicology, identification of neoplastic marker probes for DNA-diploid disease, and so many other areas where cellular systems are involved.[16–20]

II. MATERIALS AND METHODS

The magnetic stimulator system assembled is a device that includes three parts: (i) hardware, (ii) software, and (iii) a magnetic field source.

A. Hardware description

There is a microprocessor plus it contains an electronic stage for sine wave generation, which consists of a limited counter; its design allows saving the parameters in an electronic memory, according to the frequency and the time intervals of the magnetic stimulation selected for the biological sample. Furthermore, the system permits setting different time intervals for each operating frequency previously designated. The switch exit is connected to a Fourier block diagram, this converts a constant frequency value to an astern signal, so in this block, other parameters can be established, such as the power, the central value for the signal, the RMS value, and the power of harmonics in sinusoidal signals. The out frequency generated is connected to an audio amplifier

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TABLE I. Theoretical and experimental data recorded in the Rodin coil characterization.

Frequency (Hz)	Z (Ω)	Intensity current (A)	Magnetic field intensity (mT)		fem (V)
			Theoretical	Experimental	
100	6.86	1.60	3.80	4.13	0.20
800	8.78	1.25	2.97	3.05	0.85
1500	12.41	0.89	2.10	1.98	1.10
2450	18.26	0.60	1.42	1.16	1.14
2500	18.58	0.59	1.42	1.13	1.00

with power of 1000 W and 12 V of amplitude, this feeds a magnetic field source where the sample is deposited.

B. Software

Instrumentation is monitored and controlled through an algorithm performed in assembly language and installed in a microprocessor. This algorithm was designed to generate sinusoidal oscillations at different frequencies, in particular, in this work it worked at frequencies of 100, 800, 1500, 2450, and 2500 Hz in segments of 360 s each one; this frequency cycle was repeated four times in a pilot work. So, the experimental sample group was stimulated for 2 h.

C. Magnetic field resource

A Rodin system coil was assembled with two identical coils; they have an average diameter of 2.2 cm and winding of 21 turns; these two coils plugged in series, so this arrangement of coils has an electrical resistance of $R = 6.83\ \Omega$. The geometry of this magnetic stimulator device is a 12-sided polygon; furthermore, a particularity of coil geometry and its wire winding is the magnetic vortices. Such an approximation of the magnetic field sample position was estimated by using the Biot-Savart Law:

$$\vec{B}_1 = \hat{e}_r N \left[\frac{6\mu_0 I}{\pi r} \sin\left(\frac{\pi}{12}\right) \right],$$

where N is the number of coils, r is the distance from source to the sample center, and μ_0 is the magnetic permeability. But this magnetic source is an assembly of two coils, then, for the theoretical magnetic characterization was used this expression:

$$\vec{B} = \vec{B}_1 + \vec{B}_2.$$

D. Device characterization

The sinusoidal signal generated for this device was amplified through the electronic stage, then the total impedance, $|Z|$, of the circuit was measured. These data were used for calculating the electric current and then to have a theoretical estimation of the resource magnetic field. The magnetic field was also measured in the sample position with a scientific gauss meter from Magnetic Instrumentation Inc, Model 210 and sensibility from μT to T. All this information is summarized in Table I.

The theoretical and experimental behavior of the coil magnetic field is shown in Figure 1. A Pearson correlations coefficient for these registers is $r = 0.9999$, as long as, the experimental data were fitting to a first-order exponential function using Origin software. The obtained parameters, with $R^2 = 0.99$, are shown in Eq. (1):

$$MF = -0.97 + 5.32\ \exp(-3.75 \times 10^{-4} f). \quad (1)$$

On the other hand, the magnetic field and electromotive force variation are affected by frequency and impedance of the coil, these behaviors are shown in Figures 2(a) and 2(b), respectively.

In order to have an evaluation of the working of this magnetic exposure, the device was tested in a biological sample of cell culture (human lymphocytes) according the next procedure.

E. Protocol

The local bioethics committee approved this study protocol; furthermore, a written informed consent was obtained from each volunteer. In basal conditions, 20 ml of blood was donated for each volunteer.

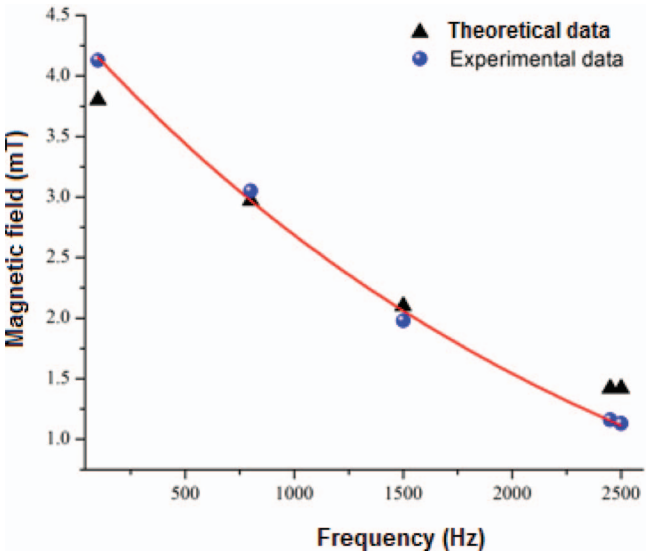

FIG. 1. Frequency responses of the magnetic field in the Rodin coil according excitation frequency.

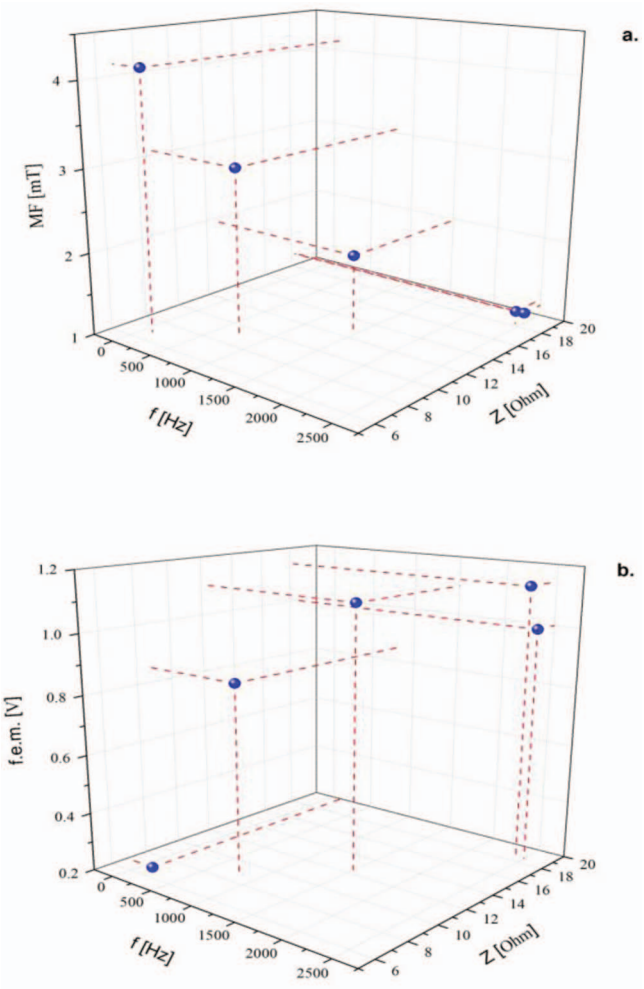

FIG. 2. This is the behavior of the magnetic field (a) and the fem (b). (a) Magnetic field vs. electrical impedance, depending on the frequency of the sinusoidal signal supplied. (b) Electromotive force vs. electrical impedance, depending on the frequency of the sinusoidal signal supplied.

1. Ferrofluid substance

A paramagnetic suspension as ferrofluid was used in order to increase the effect of magnetic field excitation in growing cells.[21–23] So, the paramagnetic Gadolinium suspension of commercial use in MRI studies was added into the cell culture of these experiments.

2. Peripheral blood mononuclear cells purification and Carboxyfluorescein Succinimidyl Ester (CFSE) labeling

Blood samples were collected from 12 subjects of 24 to 35 years old. All individuals included in this study were healthy, non-smokers, and they had no history of alcohol abuse or drug consumption. PBMCs were isolated from 20 ml of blood by conventional centrifugation in a density gradient of Ficoll-Hypaque.

After centrifugation, PBMCs were collected from the Ficoll-histopaque interface. Then, cells were washed twice with phosphate saline buffer. They were counted and the cell viability was detected by exclusion of trypan blue staining.

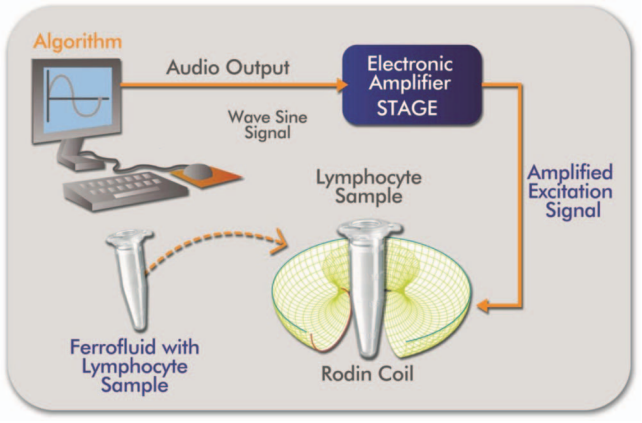

FIG. 3. Schematic setup of the magnetic excitation system applied on the prepared white blood cells.

After that, PBMCs were loaded with cell tracker dye CFSE (0.5 μM; Molecular Probes) to monitor proliferation. Next CFSE staining, 5.71 micro L of Gadolinium was added to the lymphocyte samples. Each sample was placed in a 2 ml Eppendorf tube containing the lymphocytes in RPMI-1640 medium, see Figure 3.

3. In vitro stimulation with concanavalin A and exposition to magnetic field

Two groups of samples from same donor, control, and experimental groups were prepared.

The tubes containing the PBMC plus Gadolinium were exposed to magnetic field at frequencies of 100, 800, 1500, 2450, and 2500 Hz. Each frequency was remained for 6 min, then, it changes to other of the four frequencies; this frequency cycle was repeated four times, so that the whole magnetic stimulation on the sample took 2 h. The magnetic field intensity was also changing from 1.13 to 4.13 mT, this was according to each frequency applied. Then, cells were adjusted at a concentration of 1×10^6/ml in RPMI-1640 tissue culture medium (GIBCO, Eugene, OR) supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 50 U/ml penicillin, and 50 g/ml streptomycin and cultured with or without 2.5 μg/ml of concanavalin A (Con A, Sigma Aldrich) for 72 h or 7 days at 37 °C, 100% humidity, and 5% CO_2 (in survival experiments, the cells were cultured for 20 days, as will be indicated inbrk Sec. III). The proliferation and cell survival were analyzed with a FACSCanto II flow cytometer by using the Diva software (Becton Dickinson, San Jose, CA).

III. RESULTS

A non-invasive device for stimulation with magnetic vortices in growing cells was presented. The vortices of magnetic field were programmed to work in random segments at frequencies of 100, 800, 1500, 2450, and 2500 Hz. As far as it is known, this is the first time that magnetic vortices and suspension of Gadolinium were added to the PBMC samples to improve the effect of magnetic field.

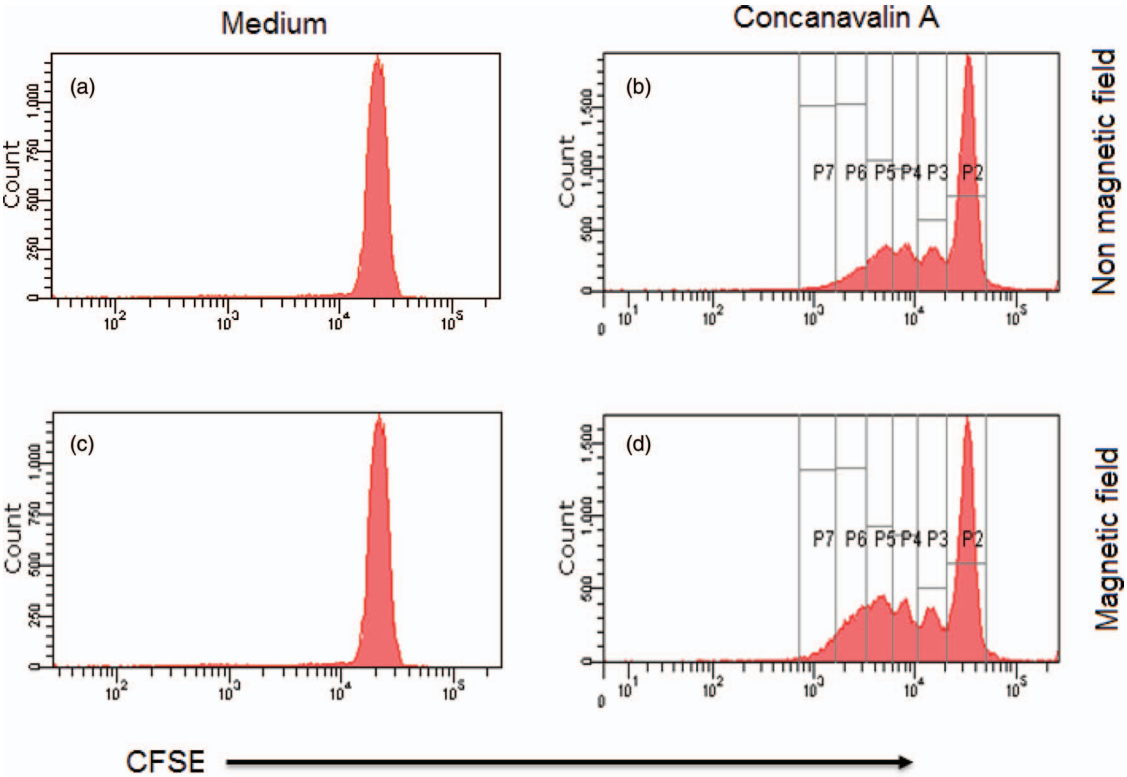

FIG. 4. Proliferation of human T cells stimulated with concanavalin A and magnetic field. (a) PBMC cultured in RPMI without concanavalin A neither magnetic field. (b) PBMC stimulated with concanavalin A but not magnetic field. (c) Concanavalin A stimulated cells without magnetic field and (d) cells stimulated with both concanavalin A and magnetic field. Percentages of cells reaching 2 (P4), 3 (P5), 4 (P6), and 5 (P7) cell divisions were significantly higher in cells stimulated with concanavalin A plus magnetic field, than those stimulated with concanavalin A alone.

In order to determine the effect of the magnetic field stimulation, a primary culture of human lymphocytes isolated from healthy donors was used. After 72 h there were no changes in cell proliferation or cell survival when the PBMCs were co-stimulated with magnetic field and concanavalin A, in comparison with those PBMCs treated only with concanavalin A (data not shown). However, after 7 days of culture, it was found that magnetic field stimulation did not induce any change on human lymphocytes without mitogenic treatment (Figure 4(c)). When human lymphocytes were co-stimulated with magnetic field and concanavalin A, an important increase in proliferating cells was detected (Figure 4(d)), thus when lymphocytes were treated with concanavalin A, but not stimulated with a magnetic field, the proliferation was observed in a lesser extent (Figure 4(c)). In Figure 4 representative plots are shown for the CFSE dilution. *In vitro* concanavalin A induced T cell proliferation, evaluated in healthy donor. The CFSE-labeled PBMCs were stimulated or not stimulated under a magnetic field, and then cultured with or without concanavalin A for 7 days and the percentage of divided cells was determined by flow cytometry.

On the other hand, when 20 days cultures of human lymphocytes were analyzed, an important increase in the number of surviving cells was detected on lymphocytes co-stimulated with magnetic field and concanavalin A (Figure 5(d)), while only few lymphocytes treated with concanavalin A but not stimulated with a magnetic field were viable after 20 days on culture (Figure 5(b)). It must be remarked that cells stimulated under a magnetic field but not treated with concanavalin A survived more than those stimulated with concanavalin A alone (Figure 5(c)).

IV. CONCLUSIONS AND DISCUSSION

It has described a new noninvasive instrumentation; this is a magnetic stimulator device, in which vortices of magnetic field are generated by a magnetic source assembly with an arrange of Rodin coil, through a sinusoidal signal and it was programmed to work at frequencies of 100, 800, 1500, 2450, and 2500 Hz. It is important to put out that these frequencies were selected after doing a pilot study and find that the effects of proliferation were evident. The effect of sinusoidal varying magnetic fields was tested on fresh human mononuclear cells stimulated *in vitro* for 7 and 20 days with a mitogen. The PBMCs were collected from healthy donors to avoid any possible alteration on cells as consequence of diseases or infections, because it is well known that different diseases and infections could alter the proliferation capacity of human cells.

These data had shown that after 72 h of culture the magnetic stimulation had not any effect in both cell proliferation and survival. However, it has been reported that exposition to electromagnetic field decreases the proliferation after mitogenic stimulation,[24] nevertheless, the very low frequency (3 Hz) and time of exposition used in other experiments were different than those used here. Controversially, others have reported an important increase of cell proliferation after electromagnetic stimulation.[25,26] Therefore, it is clear that the effect of exposition to electromagnetic field could be influenced

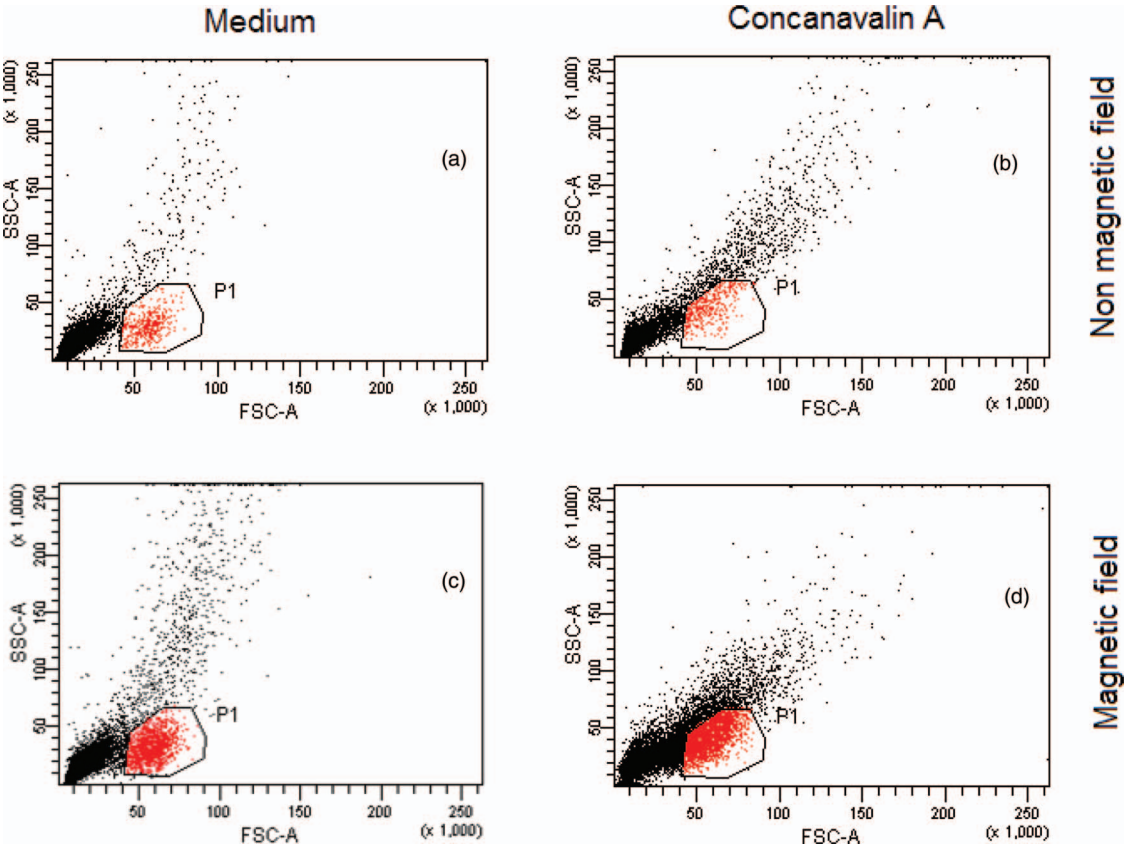

FIG. 5. Cell population and histogram obtained from flow cytometric analysis of a magnetic field excited samples from volunteer patients. P1 represents proliferating cells and P5 represents dead cells. (a) Cell divisions unstimulated with concanavalin A, (b) stimulated cells division with concanavalin A, (c) cell population unstimulated with concanavalin, and (d) cell divisions with concanavalin and magnetic stimulation.

by several experimental conditions such as, biological model, exposure system, exposure length, intensity, frequency, and pulse width.

On the other hand, it has been reported that a minimum of 6 h of exposure to electromagnetic field is necessary to induce any affect but, in most cases, a single frequency of exposure was used;[24,27] also, effects of electromagnetic fields have been observed as soon as 24 h.[26] Here, PBMC was stimulated for 2 h, in intervals of 6 min with five different frequencies and after 5 days of culture an important increase in cell proliferation on cells co-stimulated with mitogen and electromagnetic field was observed, but not on those PBMCs stimulated with mitogen alone. Moreover, the effect of electromagnetic field and mitogen also increased the survival after 20 days of cell culture. Thus, some mechanisms of cell survival have been reported to be activated by exposure to electromagnetic field such as increase in the synthesis of RNA[28] and Ca^{+2} influx and efflux have been observed,[29,30] in the same way, previously, it has been reported that cell exposition to magnetic fields increases cell survival by inhibiting apoptosis via modulation of Ca^2/influx.[14]

Finally, this is an instrumentation description of the implemented device, where segments of five frequencies, in periods of 6 min were supplied in a biological sample. Despite this work and multiple studies performed around side effects of electromagnetic field on biological model, to date there are still not enough conclusive results that indicate whether a malignant or beneficial effect is induced through electromagnetic field. Therefore, additional experiments are being carried out in our laboratories and other labs in the world in order to elucidate the intracellular mechanisms induced after electromagnetic field stimulation.

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