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Increasing survival study of kidney HEK-293T cells in magnetic field vortices and nano-fluid

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Abstract*—The behavior of the renal growth of the Human Embryonic Kidney 293 cell line (HEK-293T), previously prepared with nano-fluid base Gadolinium (Gd), and exposed to magnetic field vortices is presented. Field Intensity ranged from 1.13 to 4.23 mT was applied with frequencies segmented each six minutes in 100, 800, 1500, 2450 and 2500 Hz, during two hours. The analysis of the samples was performed using the technique of flow cytometry; it was done 72 h later magnetic stimulation. These results suggest the activation of some cellular mechanisms that induce an increase of 12.89 % (p <0.5) in cell survival. Other experiments are running in the laboratory in order to have definitive answer about the magnetic effect on growth cells.[1]

***Index Terms*—growing cells magnetic stimulation, paramagnetic nano-fluid.**

I. INTRODUCTION

Side effect of growing cells in electromagnetic fields is a scientific theme of the last years [1-4]. Particularly, correlation has been observed between pulsed magnetic fields produced by an arrangement of Helmholtz coils, the exposure time and the increase in cell proliferation in human osteosarcoma lines and normal cells in osteoblasts *in vitro* [5].

Several studies had been reported about the action of the magnetic fields on the repairing of Deoxyribonucleic acid (DNA), lymphocytes damaged by gamma radiation, cellular stress due to electromagnetic fields of extremely low frequency, changes in cell proliferation of human lymphocytes using pulsed magnetic fields and oscillations of intracellular calcium induced in human T-cell line, due to a magnetic field of 50 Hz [6-9]. Likewise, it has also focused on the search for materials for cancer therapy and the optimization of the magnetic hyperthermia [10].

Recent studies have shown that the use of nano-magnetic suspensions can increase the side effect of magnetic field stimulation. So, a Gadolinium (Gd) based suspension, commonly used in studies of magnetic resonance imaging, (MRI), Dotarem ® (0.5 mmol/L), was added to the HEK-293T cell culture in all experiments [11-14].

This is, the subject of study in this work was a cellular line (HEK-293T), and this is a cell line originally derived from epithelial cells human embryonic kidney (HEK). The 293-T variant of this cell line is a line derived from HEK-293, which also contains the Simian Vacuolating Virus 40 Tag (SV40 large T antigen), which allows replication [15].

In this work, the effect of magnetic field vortices on kidney cell cultures of HEK293T line was assessed. Nano-fluid was also added in them. The study was complemented with a survival analysis by flow cytometry technique is presented [16-19].

II. MATERIALS AND METHODS

A. HEK 293T cells

Cells were grown in DMEM complete medium (Gibco-Invitrogen) low glucose, low glutamine, with Sodium Pyruvate (110 mg/ml), supplemented with 10 % fetal bovine serum, 100 U/ml penicillin, and 100 ug/ml streptomycin for 72 hours at standard conditions of temperature, humidity and CO2 concentration (37 ° C, 100% humidity and 5 % CO2) [15].

B. Nano-fluid

Experimental group was prepared with Dotarem ® at a concentration of 0.5 mmol/ml. The dose used was 30 µL in a volume of 1.5 mL at a concentration equivalent of 0.01 mmol/mL. The commonly used dose in patients is 0.1 mmol/kg equivalent to 0.2 ml/kg.

C. Magnetic field resource

This was done with an assembly of Rodin coils, which has the particularity of produce magnetic vortices in the central region. So, the two coil windings follow a star model, they were plugged in series, each one have 21 coils, a resistance R = 6.38 Ω and diameter d = 2.2 cm.

D. Software

A computer algorithm created in Matlab Simulink platform was used to produce the sinusoidal signal, an electronic

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amplifier amplified this signal and the total value of the impedance was calculated.

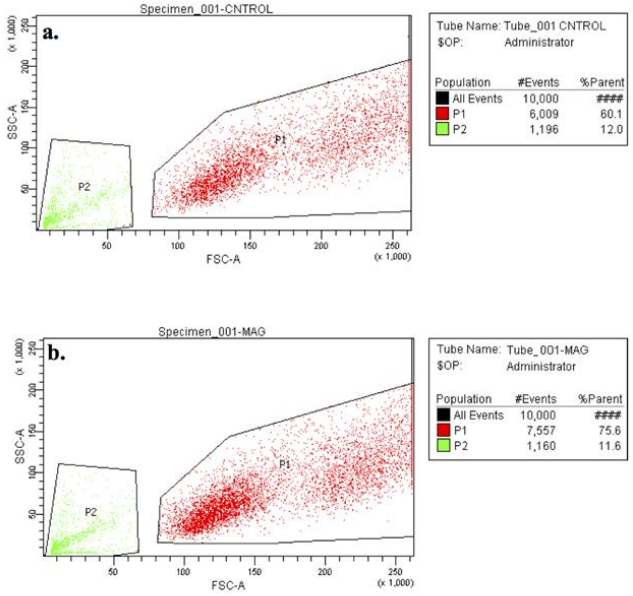

Fig 1. It is shown histograms of the control group (a.) and the experimental group (b.). There are two known populations P1 and P2, which represent the cell survival and cell in the death process.

E. Protocol

It was reviewed the monolayer formation of HEK-293T cells at the base of cell culture bottles. A washing with NaCl at 9 % was performed (saline injection). Later became trypsinization (Trypsin-EDTA, 1x, 0.05 %, GIBCO-Invitrogen) of the monolayer to achieve its dissociation. DMEM complete medium was added to the culture to neutralize the effect of trypsin and thus a cell suspension was obtained and cells were counted. The cell suspension was centrifuged and then cells were loaded with cell tracker dye CFSE (0.5 uM, Molecular Probes) to monitor proliferation. After staining with CFSE, each sample was placed in a 2 ml Eppendorf tubes containing HEK-293T cells in DMEM complete medium and 30 µl of culture DOTAREM were added to enhance the effect of the magnetic field.

F. Magnetic field stimulation

Samples of the experimental group: nano-fluid plus HEK293T underwent the magnetic field vortices at sinusoidal frequencies of 100, 800, 1500, 2450 and 2500 Hz; each frequency was applied 6 minute during 2 h, with a magnetic field changing from 1.13 to 4.13 mT [23]; control group: nano-fluid plus HEK293T that was not underwent the magnetic stimulation. When the experiment was over, $1x10^6$ cells were inoculated in a culture with Dulbecco's modified complete medium. Cell proliferation was analyzed using a flow cytometer using the FACS Canto II Diva software[1].

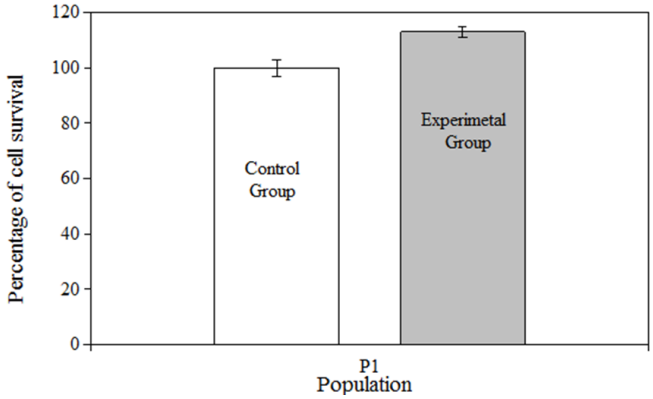

Fig 2. Survival of control samples and samples exposed to the magnetic field is shown. HEK-293T line cell was prepared with nano-fluid. P1 shows the population (survival) of 3 independent experiments performed in triplicate (P < 0.05), where a 12.89 % is the increase in cells survival.

III. RESULTS

Different parameters were evaluated (see Figure 1), Cell Size (Size-Scateter-SSC) against Cell Granularity (Foward-Side Scateter-FSC) 72 h after magnetic exposure using flow cytometry technique. In Figure 2, it is shown the survival of control samples and those exposed to the magnetic field is shown. HEK-293T line cell was prepared with nano-fluid. P1 shows the population (survival) of 3 independent experiments performed in triplicate (P < 0.05), where a 12.89 % is the increase in cells survival.

IV. DISCUSSION

As far as it is known, this is the first times that the effect of magnetic stimulation through magnetic vortices, frequency segments from 100 to 2500 Hz, and in a magnetic intensity from 1.13 to 4.13 mT is studied in kidney cell line HEK-293T.

It was shown that 72 h later to magnetic stimulation of cells with nano-fluid; the population was increased on survival and cell viability by 12.89 %.

In the literature, a large number of papers tested the effect of magnetic fields giving qualitatively and quantitatively different results depending on the characteristics of the field, some of these investigations show conflicting results reporting that as the human lymphocyte exposure to electromagnetic fields decreases proliferation by using a frequency of 3 Hz [20]. Conversely, it is also reported that the use of low frequency magnetic fields (60 Hz and 100 Gauss) and using a Helmholtz coil as a magnetic stimulator, accelerate the healing process of the skin in Balb-C mouse [21]. Similarly Cossariza reported that exposure to pulses of low frequency electromagnetic fields increases the lymphocyte proliferation in young and elderly subjects [8]. The aforementioned experiments were conducted under conditions different from those of this study: biological model, frequency, field strength, magnetic field generating coil, and exposure time, among some other conditions. However reported similar results of this investigation where

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exposure to magnetic fields decreases the degree of apoptosis in different human cell systems [22-23]. As it has seen, it was worked to determine the effect of electromagnetic radiation in different biological systems and their potential benefits or damages to them.

The results open a new modality for research; determine the mechanisms that induce or inhibit cellular level causing an increase in proliferation, survival and decreased cell death due to stimulation with controlled vortices of magnetic fields and samples in touch with nano-fluid. Due to the similarities and characteristics shared by different cell types, it can apply basic principles performed in this study and extrapolated to other human or animal cells under the same standardized conditions to determine the behavior of the same.

V. CONCLUSION

A conclusion section is not required. Although a conclusion may review the main points of the paper, do not replicate the abstract as the conclusion. A conclusion might elaborate on the importance of the work or suggest applications and extensions.

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