Proof of Concept of an ELF Magnetic Field Exposure System with Biphasic Magnetic Pulses: Effects on Human Dermal Fibroblast Proliferation

L. A. Vives*, M. Ielpi, M. T. Sosa, M. Risk and O. Patiño

***Abstract*— The aim of this work was to validate the usefulness of an Extremely Low Frequency (ELF) magnetic field exposure system with a magnetotherapy device of common use in clinical practice that generates Biphasic Magnetic Pulses (BMP), in order to study the effects of the BMP on the proliferation of Human Dermal Fibroblasts (HDF). In that regard, HDF were exposed 2h in the morning and 2h in the afternoon for 2 days to BMP of 1.41mT peak value at 5, 10, 25Hz continuous and 50Hz intermittent (2s on/ 1.5s off). MTT assay was performed to assess proliferation. The 10Hz BMP showed a significant decrease in proliferation of 6.6% ($p = 0.001$) with respect to controls, but no significant changes in proliferation were seen with the other BMP. In order to analyze whether these results could be related to the exposure protocol, a 50Hz power line intermittent signal (1s on/ 1s off) was generated and tested but exposure time was increased to 48h to cover the complete cells doubling time. A significant increase in proliferation of 9% ($p < 0.001$) was found in this case.**

The results validate the in vitro exposure system for its use with the BMP. Though the MTT proliferation assay alone is not enough to make definitive claims, the results might indicate that the exposure time plays a key role in the outcome of the experiments. Therefore, special attention should be paid to the exposure time on in vitro protocols and how they relate to in vivo experiments and current treatments.

***Index Terms*— biphasic magnetic pulses, extremely low frequencies, human dermal fibroblast, in vitro exposure, proliferation assessment.**

I. Introduction

Biologicalxs effects of Extremely Low Frequency (ELF) electromagnetic fields have been investigated for more than forty years. Probably the most reported effect of ELF Magnetic Fields (MF) on cells is the change in the proliferation rate, where both inhibition and stimulation have been observed, depending on cell type and exposure conditions [1] - [6].

L. A. Vives is with the Institute of Scientific and Technical Research for Defense (CITEDEF), Buenos Aires, B1603 Argentina, (e-mail: lvives@citedef.gob.ar).

M. Ielpi, M. T. Sosa and O. Patiño are with the Hospital Italiano de Buenos Aires, Buenos Aires, C1199 Argentina (e-mail: marcelo.ielpi@hospitalitaliano.org.ar; malena.sosa@hospitalitaliano.org.ar; osvaldojpatino@gmail.com).

M. Risk is with the Instituto de Medicina Traslacional e Ingeniería Biomédica (IMTIB) - CONICET- Instituto Universitario del Hospital Italiano - Hospital Italiano de Buenos Aires, Buenos Aires, C1199 Argentina (e-mail: marcelo.risk@hospitalitaliano.org.ar).

Though still there are no generally accepted mechanisms of action and dose-effect relationship, commercially available bone growth stimulators and different magnetotherapy devices have been developed through these years to treat several pathologies like bone edema, skin ulcers, pseudo arthrosis, and chronic pain just to mention some of them. Different pathologies are usually treated with different MF signals. Instances of these signals are: sinusoidal fields, combined fields (DC+AC), pulsed electromagnetic fields (PEMF) and intermittent fields (IONOFF) [7].

Magnetotherapy has become a common practice in many physical therapy centers around the world. According to information obtained from informal conversations with physical therapists of the Hospital Italiano de Buenos Aires, treatments consist of ten or more exposure sessions to MF, that may last from 30min to 2h, though in certain cases (e.g. pseudo arthrosis) patients generally rent the device and sessions can last as long as 8h a day for 3 or 4 months.

In Argentina, a magnetotherapy device of common use is the Magnetherp 330 (Meditea Electromedica, Buenos Aires, Argentina), which generates continuous and IONOFF Biphasic Magnetic Pulses (BMP) of half sine shape (Fig. 1) for treating different pathologies. Though high intensity (0.5-1T) BMP of half sine shape are typically associated with transcranial magnetic stimulation [8], this kind of BMP of much lower intensities are seldom related to magnetotherapy. The objective of this work was to validate the usefulness of an ELF-MF exposure system, with the BMP generated by the Magnetherp 330, exposing Human Dermal Fibroblasts (HDF) and assessing possible changes in proliferation, given that, to the best of our knowledge, these signals have rarely been used in in vitro ELF-MF exposure experiments and no experiments on cells were published with this specific device. Also, HDF play an essential role during cutaneous wound healing and in bioengineering of skin [9]. The exposure protocol was designed considering the use of the device in an actual treatment situation. HDF were obtained from skin punch biopsy of a healthy donor with informed consent, isolated and cultured.

This study is part of the project Bioelectromagneto by The Institute of Scientific and Technical Research for Defense (CITEDEF) and the Instituto de Medicina Traslacional e Ingeniería Biomédica (IMTIB), which is focused on the effect of ELF-MF on cells.

II. MATERIALS AND METHODS

A. Magnetic Fields Signals

The Magnetherp 330 can generate continuous and IONOFF BMP of the following frequencies: 5, 10, 25 and 50Hz. The basic pattern of the signal consists of 2.5 milliseconds (ms) positive half sine, 7.5ms null, 2.5ms negative half sine and another 7.5ms null (Fig.1). Thus, in order to generate the 5, 10, 25 and 50Hz signals, this basic pattern is repeated every 200, 100, 40 and 20ms respectively (Fig. 2).

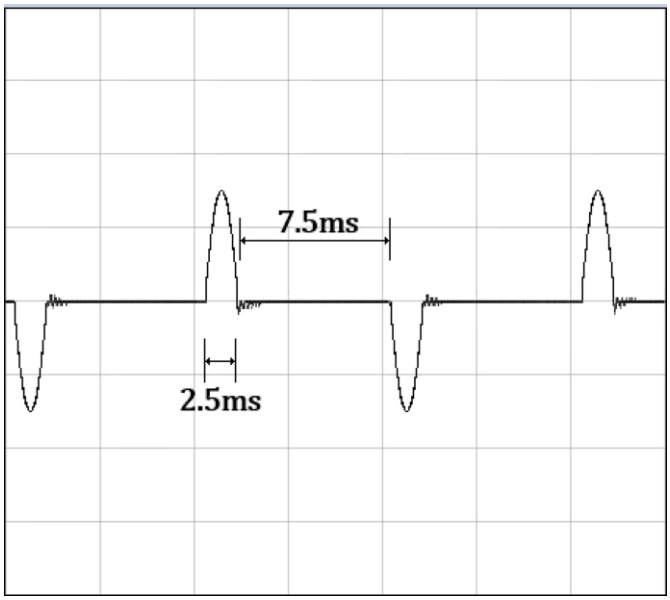


Fig. 1. Main pattern of the biphasic magnetic pulses.

The IONOFF mode is generated by turning the signal on for 2s and off for 1.5s (Fig. 2D). The authors found no information about the theoretical or empirical basis for the use of these signals, nor was it provided by the manufacturer.

Fast Fourier Transform (FFT) was performed on each signal in continuous mode using Microsoft Excel (Microsoft Corporation, Redmond, WA) to analyze the frequency composition. Two things are important to point out from this analysis. The first is that the maximum amplitude is not located in the fundamental frequency for the 5, 10, and 25Hz signals but in 50Hz for all of them, which is related to the time span of the basic pattern (20ms). The second is that there seems to be no harmonics for multiples of 100Hz. Figure 3 shows the FFT performed on the continuous 10Hz BMP. The small peaks present at multiples of 100Hz, as well as a little shift of the zeros in 400 and 500Hz, could be due to spectral leakage caused by finite windowing of the data.

Considering the different settable treatments that the device offers, four of these signals were chosen for this work: 5, 10, 25Hz continuous BMP, and 50Hz IONOFF BMP (Fig. 2). Also a 50Hz power line IONOFF signal (1s on/ 1s off) was generated by the authors and used to extend the exposure time to 48h, given that it was not possible to reconfigure the Magnetherp 330 for this purpose.

B. Exposure System

An exposure system was designed and developed for project Bioelectromagneto. It is based on two identical sets of four magnetically shielded Lee-Whiting coils, placed in a Forma Scientific 3194 incubator (Thermo Fisher Scientific, Waltham, MA), which can generate magnetic flux densities (B fields) up to 1mT root mean square (rms) with no active cooling system and up to $3mT_{rms}$ with it. The B Field-to-current ratio is 699.4μT/A. The double-wrapped windings, with twisted pairs, allow for the use of each set of coils either as exposure or control, with no detectable parasitic B field in the control. The artifacts have also been analyzed: the B field in the center of the sham control chamber is about $1\mu T_{rms}$ for a maximum of $3mT_{rms}$ in the exposure chamber, the parasitic incident electric fields are less than 1V/m, the temperature difference between sham and exposure chamber is less than or equal to 0.2°C, and the typical vibration difference between sham and exposure is less than $0.1m/s^2$. The system was tested and published [10].

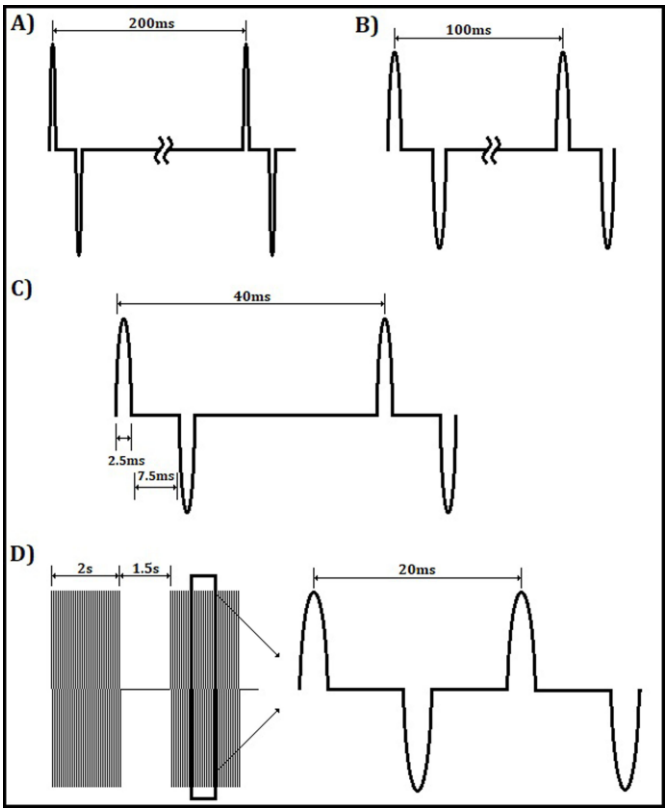


Fig. 2. Biphasic magnetic pulses of different frequencies: (A) 5Hz, (B) 10Hz, (C) 25Hz and (D) 50Hz IONOFF (2s on / 1.5s off).

As mentioned above, BMP were generated with the Magnetherp 330 connected to the coils system. During exposures, peak current was controlled by measuring the voltage drop across a resistor (0.8Ω, 2W), connected in series with the coils, with an oscilloscope, DSOX2002A (Agilent Technologies, Santa Clara, CA). The 50Hz power line IONOFF signal was generated using an autotransformer, Variostat (Laboratorios Variotron, Buenos Aires, Argentina) plugged to the power line socket and turning on and off the

signal with a relay controlled by a 555 timer circuit. The complete exposure system is shown in figure 4.

C. Exposure Protocol

The Magnetherp 330 allows for setting the MF intensity between 0.2mT and 20mT, and is delivered to the patient either by placing a small flat coil (diameter: 13.5cm, height: 2,5cm) on the treated zone or inserting the limb into a solenoid coil (diameter: 22.5cm, length: 19cm). During an actual treatment, the MF intensity can differ substantially from the value set in the device. For instance, in the case of the flat coil, the intensity measured at the center, over the plastic case surface, where the maximum field value is found, falls 85% at 5cm from that point along the symmetry axis. In the case of the solenoid, the intensity decreases to a 60% of the maximum close to the edges. Taking this fact into account, and considering that the patient is usually exposed to lower intensities than those set, the MF intensity selected for this work was 1.41mT peak value, which corresponds to the peak value of a sine wave of $1mT_{rms}$, and the highest field our exposure device can generate without an active cooling system.

The maximum exposure time that can be set in the Magnetherp 330 is one hour. The exposure time per session is usually decided by each therapist depending on his/her experience, but is never longer than 2h and no more than 2 sessions per day. According to this, and in order to test the signals under similar circumstances, experiments were performed exposing the cells 2h in the morning and 2h in the afternoon for 2 days (the operator had to reset the device after one hour to achieve the 2h exposure). This protocol was compared with a 48h exposure protocol performed with a 50Hz power line IONOFF signal (1s on/ 1s off).

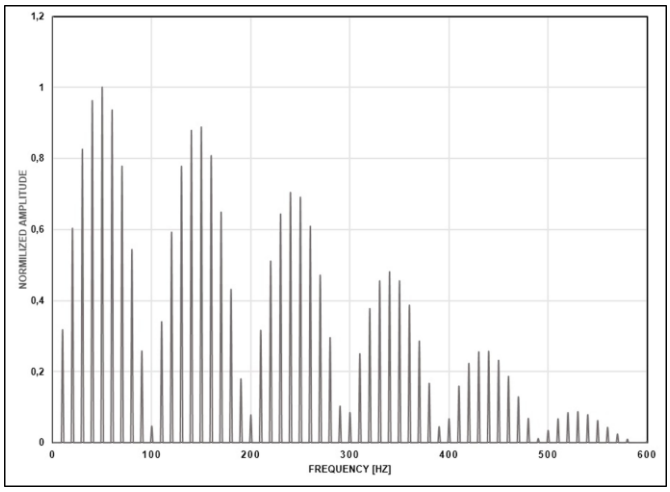


Fig. 3. FTT performed on the continuous 10Hz BMP.

D. Isolation and Culture of Human Dermal Fibroblasts

Skin biopsy was obtained from a healthy 40-year-old female donor with informed consent. The protocol was approved by the Institutional Research Protocol Ethics Committee (Hospital Italiano de Buenos Aires, Protocol Number: 2648). Tissue was washed with Hank's Balanced Salt Solution (HBSS) (Sigma-Aldrich, St. Louis, MO). Dermis was removed with sterilized scissors and fragmented into $1mm^3$ pieces. Explants were cultured on 60mm diameter petri dishes (Nunc, Roskilde, Denmark) with 2mL Dulbecco's Modified Eagle Medium (DMEM) (Gibco, Billings, MT), supplemented with 100U/mL penicillin, 100µg/mL streptomycin, 0.25µg/mL amphotericin B (ATB) (Gibco) and 10% Fetal Bovine Serum (FBS) (Internegocios, Buenos Aires, Argentina). Cultures were performed in a humidified atmosphere with 5% CO2 at 37°C.


Fig. 4. Exposure system.

When cell confluence was reached, tissue explants were removed from the dish and cells were subcultured for magnification. The dish was treated with a 0.25% trypsin solution (Gibco) for 3min. After neutralizing the trypsin, cells were centrifuged at 1100rpm for 10min, resuspended, and plated in T75 flasks (Nunc) with 10mL of culture medium. Cells were quantified using the Trypan blue (Gibco) exclusion method.

Growth curve was analyzed for cultures for three passages between passage 5 and 12. Cells were counted every 24h using a hemocytometer. The average doubling time measured was 36h after cells attachment (24h after seeding). Standard

deviation of the cell counts grew abruptly after 72h from seeding. Accordingly, for the exposure experiments we decided to perform proliferation assay at 72h after seeding and not beyond that time.

E. Proliferation Assay Protocol

Cells were seeded in eight 30mm diameter petri dishes (Nunc), $50x10^3$ cells per dish, with 2mL of medium each (DMEM 90%, FBS 10%, ATB 1% of total culture medium). Four were used as exposed and four as controls. Dishes were placed in the coils system and left 24h for cells to attach before exposures started. For the first four signals, exposures were performed for 2 days, 2h in the morning and 2h in the afternoon. For the fifth signal, exposures lasted 48h. In all cases, 72h after seeding dishes were taken out of the coils and MTT proliferation assay was performed.

At first, medium was discarded from cell cultures. 300µL of 5mg/ml MTT (Sigma-Aldrich) and 300µL of fresh medium were added to each culture. All cultures were then incubated for 3h at 37ºC. After that time, supernatant was discarded and 500µL of Dimethyl-Sulfoxide (DMSO) (Sigma-Aldrich) were added to each culture. Dishes were wrapped in tin foil and shaken on an orbital shaker for 15min to fully dissolve the blue crystals. Three samples of 150µL were taken from each culture to triplicate the reading. Correspondingly, 12 wells readings were obtained for control and exposed cells per experiment. Absorbance (Optical Density), which can be directly correlated to cell number, was measured at 492nm, using a 96-well microplate reader Stat Fax-2100 (Fisher Bioblock Scientific, Newburgh, NY) given that it was the highest wavelength available in the reader. MTT assay using this wavelength was performed by different authors [11]-[14].

F. Statistical Analysis

To facilitate comparisons, in each experiment, the absolute absorbance values of the exposed and control readings were normalized (i.e., divided) by the average of the controls, hence the average of the controls was always identical to 1 and the proliferation of the exposed cells are reported relative to control [6]. All experiments were repeated three times and experimental data for each MF signal were pooled and averaged to produce each proliferation measurement. The results are expressed as the mean ± standard error of the mean (SEM). A Two-Sample t-Test was performed to determine whether there was a significant difference between the means of the exposed and control cells. P-values less than 0.05 were considered statistically significant. The statistical analysis was performed using Minitab 17 (Minitab, State college, PA).

III. RESULTS

All experiments were performed thrice, swapping the condition of both coils systems (exposure and control) each time. Figure 5 shows the proliferation relative to control averaged over three experiments. The continuous 10Hz BMP showed a significant decrease in proliferation of 6.6% ($p = 0.001$) with respect to control, but no significant changes in proliferation were seen with the other BMP generated with the Magnetherp 330 (p-values ranged from 0.3 to 0.78). A significant increase in proliferation of 9% ($p < 0.001$) was found in the case of the 50Hz power line IONOFF (1s on/ 1s off) signal. No morphological changes (size and shape) were observed between exposed and controls, or within the exposed dishes in any of the experiments.

IV. DISCUSSION

As introduced above, the most reported effect of ELF-MF on cells is the change in the proliferation rate. In addition, stimulation and inhibition were reported, and both effects may have promising clinical use. Nevertheless, mechanisms of action and dose-effect relationship remain unclear, probably due to the number of variables that might play an important role in the outcome of experiments such as B field intensity, frequency content, waveform, exposure time, the time between exposures, and in the case of IONOFF signals, the on and off span.

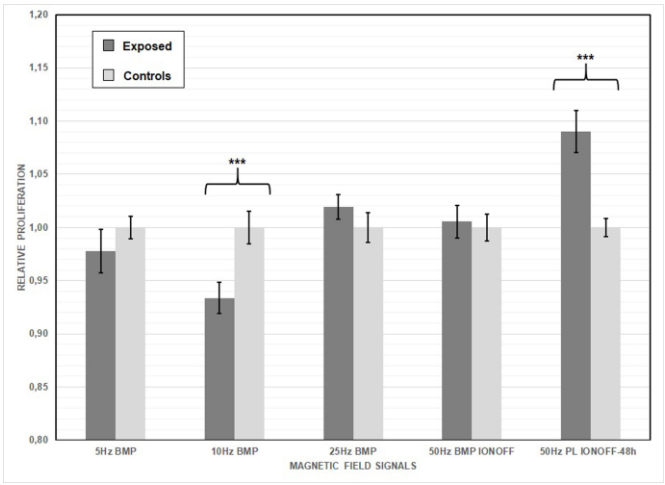


Fig. 5. Relative proliferation of the control and exposed cells for 5Hz, 10Hz, 25Hz BMP, 50Hz BMP IONOFF (2s on/ 1.5s off) and 50Hz Power Line IONOFF (1s on/ 1s off) 48h. Bars are ± SEM for three experiments. *** ($P \leq 0.001$).

More than forty years have passed since the first work with PEMF applied to stimulate bone growth [15] (which were in essence biphasic, but not half sine pulses) and many magnetotherapy devices have been developed and commercialized since then. The Magnetherp 330 has never been tested before in in vitro experiments. The exposure protocol was designed according to its regular clinical use. Under this protocol, only the 10Hz BMP showed a significant effect on proliferation (6.6% decrease). The reason for this outcome is unknown. Though all BMP applied have a wide content of harmonics, the maximum amplitude in the frequency spectrum is yet located in 50Hz, due to the time span of the BMP pattern (20ms). Had the effect been produced by the 10Hz BMP harmonics content, then the 5Hz BMP could have shown a similar effect because it also contains those harmonics, unless the harmonics that are only present in the latter could have diminished the effect. Another reason for not detecting a significant change in proliferation with the other signals could have been the exposure time. 2h in the morning and 2h in the

afternoon for 2 days may have not been enough time to stimulate the cells in all cases. This idea can be supported by the fact that we detected a 9% significant increase in proliferation with the 50Hz power line IONOFF signal with 48h exposure, though it is also true that we could not generate exactly the same 50Hz BMP IONOFF generated by the device.

According to our results, this exposure system is useful to perform in vitro experiments with the BMP generated by the Magnetherp 330, however mimicking an actual treatment exposure protocol might not be the best approach for in vitro experiments and exposing cells during the complete doubling time could be a better exposure time choice to detect MF effects and thus be able to study mechanisms of action.

V. Conclusions

The usefulness of the exposure system here presented, for testing the BMP generated by the Magnetherp 330, was validated for the first time in an in vitro experiment. Even though the MTT proliferation assay alone is not enough to make definitive claims, our results might indicate that the continuous 10Hz BMP could reduce cell proliferation and could be an interesting signal to be tested by those researchers that work with cancer cells [6] [14]. Likewise, a 50Hz power line IONOFF (1s on/ 1s off) signal might increase cell proliferation and could be a useful MF signal to treat skin ulcers and pseudo arthrosis [4] [16] [17]. Considering the similarities between the frequency composition and IONOFF spans of the 50Hz BMP and the 50Hz power line fields, the results obtained with both signals and protocols suggest that the exposure time might play a key role in the outcome of the experiments. Therefore, special attention should be paid to the exposure time on in vitro protocols and how they relate to in vivo experiments and actual treatments. Our results are in line with the literature in that the same cells can respond differently to different MF signals and exposure times.

Acknowledgment

The authors want to acknowledge for their assistance the following partners of the Hospital Italiano de Buenos Aires: Bioc. Patricia Sorroche and Bioc Leticia Yamamoto (Central Laboratory) and Dr. Marcela Bolontrade (IMTIB). The authors are also grateful to Dr. Juan Mucci of the IIB (Instituto de Investigaciones Biotecnológicas, Universidad Nacional de San Martín) for providing us with the MTT and Sebastián Fernandez Ellero for his help with the statistical analysis.

This work was supported in part by the Fundación Florencio Fiorini (Argentina) under Grant "Biomedical Sciences Research – 2015}.

References

[1] S. M. Ross, "Combined DC and ELF Magnetic Fields Can Alter Cell Proliferation," *Bioelectromagnetics*., vol. 11, no. 1, pp. 27-36, 1990.

[2] F. Pezzetti, M. De Mattei, A. Caruso, R. Cadossi, P. Zucchini, F. Carinci, G. C. Traina and V. Sollazzo, "Effects of Pulsed Electromagnetic Fields on Human Chondrocytes: An In Vitro Study," *Calcif Tissue Int*., vol. 65, no. 5, pp. 396-401, Nov. 1999.

[3] G. Olsson, I. Y. Belyaev, T. Helleday and M. Harms-Ringdahl, "ELF magnetic field affects proliferation of SPD8/V79 Chinese hamster cells but does not interact with intrachromosomal recombination," *Mutat Res*., vol. 493, no. 1–2, pp. 55–66. Jun. 2001.

[4] G. Hannay, D. Leavesley and M. Pearcy, "Timing of Pulsed Electromagnetic Field Stimulation Does Not Affect the Promotion of Bone Cell Development," *Bioelectromagnetics*., vol. 26, no. 8, pp. 670-676, Sep. 2001.

[5] C. Seeliger, K. Falldorf, J. Sachtleben and M. Van Griensven, "Low-frequency pulsed electromagnetic fields significantly improve time of closure and proliferation of human tendon fibroblasts," *Eur J Med Res*, vol. 19, no. 1, pp. 37-42, Jul. 2014.

[6] L. Makinistian, E. Marková and I. Belyaev, "A high throughput screening system of coils for ELF magnetic fields experiments: proof of concept on the proliferation of cancer cell lines," *BMC Cancer*., vol. 19:188, Feb. 2019.

[7] M. Markov, "XXIst century magnetotherapy," *Electromagn Biol Med*., vol. 34, no. 3, pp.190-196, Sep. 2015.

[8] I. Delvendahl, N. Gattinger, T. Berger, B. Gleich, H. R. Siebner and V. Mall, "The Role of Pulse Shape in Motor Cortex Transcranial Magnetic Stimulation Using Full-Sine Stimuli," *Plos ONE*., vol. 9, no. 12, pp. 115-247, Dec. 2014.

[9] M. A. Kisiel and S. Klar, "Isolation and Culture of Human Dermal Fibroblasts," in *Skin Tissue Engineering*, New York, NY, USA: Springer Science + Business Media, 2019, pp. 71-78, May.

[10] L. Vives, J. Balsalobre, T. Monteiro, J. García Díaz, G. Liponetzky, M. Ielpi, N. Dalmas Di Giovanni, "ELF Magnetic Field Exposure System for In Vitro Studies Based on Lee-Whiting Coils," *Bioelectromagnetics*., vol. 41, no. 5, pp. 382-391, Jun. 2020.

[11] H. Huang, H. Lin, X. Zhang and J. Li, "Resveratrol reverses temozolomide resistance by downregulation of MGMT in T98G glioblastoma cells by the NF-κB-dependent pathway," *Oncol Rep*., vol. 27, no. 6, pp. 2050-2056, Jun. 2012.

[12] M. Zhang, X. Li, L. Bai, K. Uchida, W. Bai, B. Wu, W. Xu, H. Zhu and H. Huang, "Effects of Low Frequency Electromagnetic Field on Proliferation of Human Epidermal Stem Cells: An In Vitro Study," *Bioelectromagnetics*., vol. 34, no. 1, pp. 74-80, Jan. 2013.

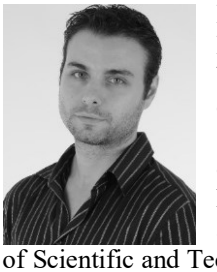
[13] G. Z. An, H. Xu, Y. Zhou, L. Du, X. Miao, D. P. Jiang, K. C. Li, G. Z. Guo, C. Zhang and G. R. Ding, "Effects of Long-Term 50Hz Power-Line Frequency Electromagnetic Field on Cell Behavior in Balb/c 3T3 Cells," *Plos ONE*., vol. 10, no. 2, pp. 1-13, Feb. 2015.

[14] F. Wang, Y. Mao, Q. You, D. Hua and D. Cai, "Piperlongumine induces apoptosis and autophagy in human lung cancer cells through inhibition of PI3K/Akt/mTOR pathway," *Int J Immunopathol Pharmacol*., vol. 28, no. 3, pp. 362-373, Aug. 2015.

[15] C. A. Bassett, R. J. Pawluk and A. A. Pilla, "Augmentation of bone repair by inductively coupled electromagnetic fields," *Science*., vol. 184, no. 4136, pp. 575-577, May. 1974.

[16] O. Patiño, D. Grana, A. Bolgiani, G. Prezzavento, J. Miño, A. Merlo and F. Benaim, "Pulsed electromagnetic fields in experimental cutaneous wound healing in rats," *J Burn Care Rehabil*., vol. 17, no. 6, pp. 528-531, Mar. 1996.

[17] A. Athanasiou, S. Karkambounas, A. Batistatou, E. Lykoudis, A. Katsaraki, T. Kartsiouni, A. Papalois and A. Evangelou, "The effect of pulsed electromagnetic fields on secondary skin wound healing: an experimental study," *Bioelectromagnetics*., vol. 28, no. 5, pp. 362-368, Jul. 2007.



Leandro A. Vives received the degree in Electronics Engineering from the University of Buenos Aires in 2005 and is currently doing his Ph.D. in applied sciences and engineering at the University of San Martín (Argentina). Since 2004 he has been working in the area of Antennas and Non Ionizing Radiation in the Institute of Scientific and Technical Research for Defense (CITEDEF). He is currently an Associate Professor in Electromagnetics and Bioelectromagnetics at the Instituto Universitario del Hospital Italiano de Buenos Aires (IUHIBA). Eng. Vives is a member of the Bioelectromagnetics Society (BEMS).

Marcelo Ielpi received the degree in Biochemistry from the University of Morón (Argentina) in 2001. He joined the Hospital Italiano de Buenos Aires to assist in the area of cell culture in 2001 and since 2008 he is in charge of cell culture and cryopreservation. He is also part of the pediatric unrelated donor HSC transplant team and the pediatric halogen and autologous HSC transplant team.

Malena T. Sosa graduated as a hematology technician in 2012 at the Instituto Superior de Tecnicaturas para la Salud del Gobierno de la Ciudad de Buenos Aires, and is currently studying Medicine at the University of Buenos Aires. She works at the Hospital Italiano de Buenos Aires as an assistant in the area of cell culture and cryopreservation and as a member of the Support Staff for Research and Development Career (CPA) at the Consejo Nacional de Investigaciones Cientificas y Tecnicas (CONICET).

Marcelo Risk holds a PhD from the Faculty of Medicine of the University of Buenos Aires, a ScD in Bioengineering from the National University of Córdoba, an MBA from the University of Palermo and a degree in Electronics Engineering from the National Technological University (Argentina). He is currently director of the Instituto de Medicina Traslacional e Ingenieria Biomedica (IMTIB), associated with the Consejo Nacional de Investigaciones Cientificas y Tecnicas (CONICET), Instituto Universitario del Hospital Italiano de Buenos Aires and the Hospital Italiano de Buenos Aires, Argentina, director and full-time Professor, in the Biomedical Engineering program at IUHIBA.

Osvaldo Patiño received the degree in Physiotherapy in 1977 and a PhD in Medicine in 2002, both from the University of Buenos Aires. He has worked for more than forty years as physiotherapist, research scientist and university Professor. He is currently the honorary chief of the Physical Medicine and Rehabilitation department of the Hospital Italiano de Buenos Aires and a professor in the Universidad de Gran Rosario (UGR). Dr. Patiño is a member of the American Association of Physical Therapy, the European Society for Shoulder and Elbow Rehabilitation and the International Society for Medical Shockwave Treatment among other societies.