

# Cellular and molecular effects of electromagnetic radiation and sonic waves

## AUTHORS:

Patrícia Froes Meyer<sup>1</sup>  
Oscar Ariel Ronzio<sup>2</sup>  
Adenilson de Souza da Fonseca<sup>3</sup>  
Sebastião David Santos-Filho<sup>3</sup>  
Mario Bernardo-Filho<sup>3,4</sup>

## AFFILIATIONS:

<sup>1</sup>Postgraduate Program in Health Science, Rio Grande do Norte Federal University, Natal, Brazil

<sup>2</sup>Physical Agents Laboratory, Maimonides University, Buenos Aires, Argentina

<sup>3</sup>Biophysics and Biometry Department, Roberto Alcántara Gomes Biology Institute, Rio de Janeiro State University, Rio de Janeiro, Brazil

<sup>4</sup>Research Coordination, National Cancer Institute, Rio de Janeiro, Brazil

## CORRESPONDENCE TO:

Sebastião David Santos-Filho

## EMAIL:

sdavidofilho@gmail.com

## POSTAL ADDRESS:

Departamento de Biofísica e Biometria, Instituto de Biologia Roberto Alcántara Gomes, Universidade do Estado do Rio de Janeiro, Avenida 28 de Setembro, 87, Vila Isabel, 20551-030, Rio de Janeiro, Brazil

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Electromagnetic radiation (in the form of pulsed magnetic fields, radiofrequency and intense pulsed light) and mechanical agents (such as sonic waves) have been used in physical therapy. The aim of this study was to assess the effects of low-intensity magnetic fields, sonic and radiofrequency waves, and intense pulsed light on the survival of *Escherichia coli* cultures and on the electrophoretic mobility of plasmid DNA. Exponentially growing *E. coli* AB1157 cultures and plasmid DNA samples were exposed to these physical agents and 0.9% NaCl (negative control) and SnCl<sub>2</sub> (positive control) solutions. Aliquots of the cultures were diluted and spread onto a solidified rich medium. The colony-forming units were counted after overnight incubation and the survival fraction was calculated. Agarose gel electrophoresis was performed to visualise and quantify the plasmid topological forms. The results suggest that these agents do not alter the survival of *E. coli* cells or plasmid DNA electrophoresis mobility. Moreover, they do not protect against the lesive action of SnCl<sub>2</sub>. These physical agents therefore had no cytotoxic or genotoxic effects under the conditions studied.

## Introduction

Physical therapy devices used for the treatment of aesthetic disorders<sup>1,2</sup> such as facial acne, can emit sonic and ultrasonic waves and electromagnetic radiation at an extremely low frequency as well as radiofrequency, light and infrared radiation.<sup>2,3</sup> Low frequency pulsed electromagnetic fields (PEMF) could be used to treat diseases characterised by pain, inflammation and regeneration. Biological effects on the organs and body systems associated with the energies generated by these sources have been reported, but the findings remain inconsistent.<sup>4</sup> Beneficial effects of electromagnetic fields on bone metabolism and hydroxyapatite osteointegration, suggesting osteogenesis stimulation, have been described.<sup>5</sup>

Some authors have suggested that audible sonic waves could interact with proteins, moving them to the lymphatic system, as in the bioresonance phenomenon.<sup>4</sup> According to this phenomenon, proteins move to the lymphatic system as a result of the harmonics created by the sonic waves, thereby exiting the extracellular compartment.<sup>6</sup>

Effects associated with radiofrequency are related to heating of the tissues to 50 °C, at which cell death is induced by protein coagulation – an effect that could be useful in the treatment of tumours.<sup>7</sup> Effects on the treatment of muscle and articular injuries have been reported with the use of radiofrequency. Radiofrequency has been used for aesthetic purposes and some authors have suggested a thermal action in deep tissues, promoting collagen denaturalisation and neocollagenogenesis.<sup>8,9</sup>

Intense pulsed light (IPL) sources have been used to treat abnormal scarring,<sup>10</sup> burn sequelae, hyperchromia and benign vascular lesions.<sup>11,12</sup>

Stannous chloride (SnCl<sub>2</sub>) is the most widely used reducing agent in nuclear medicine for labelling cellular and molecular structures of biological interest. It is used with technetium-99m in single-photon emission computed tomography. However, it has been shown that SnCl<sub>2</sub> is cytotoxic and genotoxic.<sup>13,14</sup> In bacterial cultures and plasmid DNA, SnCl<sub>2</sub> appears to induce damage through oxidative mechanisms related to free radical generation.<sup>15,16</sup> Data from studies on *Escherichia coli*, deficient in DNA repair mechanisms, suggest that this chemical agent could induce lesions in DNA.<sup>14,16</sup>

Although extremely low-frequency electromagnetic fields, sonic, radiofrequency and IPL devices are used in therapeutic practice, there have been few studies on their biological effects. The aim of this study was therefore to evaluate the effect of these sources of energy on the survival of *E. coli* and on the electrophoretic mobility of plasmid DNA.

## Materials and methods

### Physical agent exposure

A PEMF device was used (700 Ohms, 110 V, 60 Hz) to generate a polarised electromagnetic field (north and south). Bacterial cultures and DNA samples were exposed at both magnetic poles (5 mT, 30 min).

Sonic waves were generated by a Bioressonance® device configured at 3.3 KHz. Bacterial cultures and DNA samples were exposed for 20 min. The piezoelectric emitters were coupled to the samples with ultrasonic gel.

The PEMF and Bioressonance® devices are archetypes developed and tested by Oscar Ronzio, a physical therapist at Maimonides University in Buenos Aires, Argentina.

Radiofrequency, at a frequency of 550 KHz, was applied for 5 min using an electrical capacitive transference device (Tecatherap-VIP®, VIP Electromedicina®, Buenos Aires, Argentina). Bacterial cultures and DNA samples were placed between two equidistant (30 mm) rubber covers with carbon electrodes.

Bacterial cultures were exposed to one pulse (0.01 s, 3–7 J/cm<sup>2</sup>, 500–1200 nm) and DNA samples were exposed to one and two pulses (at the same specifications) of intense pulsed light using an IPL device (Radiance®, Tel Aviv, Israel).

### Bacteria inactivation

*Escherichia coli* AB1157, a wild-type strain to repair DNA damage, was used in this study. Exponentially growing bacterial cultures in rich medium were centrifuged at 2000 rpm for 10 min and suspended in saline solution (0.9% NaCl). Samples of these suspensions (1.0 mL) were exposed to each physical agent. Unexposed samples treated with saline were used as a negative control and those incubated with SnCl<sub>2</sub> (25 µg, 60 min) were used as a positive control. The cultures were diluted and spread on Petri dishes containing solidified rich medium. After overnight incubation at 37 °C, the colony-forming units were counted and the survival fractions were calculated by dividing the number of viable cells obtained per millilitre after treatment (with physical agents or SnCl<sub>2</sub>) by the number of viable cells before treatment.

### Analysis of DNA mobility alterations

Samples of pBSK plasmids (200 ng) were obtained by the alkaline method<sup>17</sup> and exposed to the physical agents as described. Unexposed plasmid samples treated with saline were used as a negative control and unexposed plasmid samples incubated with SnCl<sub>2</sub> (200 µg, 40 min) were used as a positive control. Aliquots of each sample were then mixed with loading buffer (0.25% xylene cyanol, 0.25% bromophenol blue, 30% glycerol) and 0.8% agarose gel electrophoresis (8 V/cm) was performed in Tris-acetate-EDTA buffer (pH 8.0). Gels were then stained with ethidium bromide (0.5 µg/mL), and the DNA bands were visualised by fluorescence in an ultraviolet transilluminator system. The gel images were digitalised (Kodak Digital Science 1d, EDAS 120, Rochester, NY, USA) and the bands were semiquantified using the Gimp computer program. Plasmid conformations were quantified as either Form I supercoiled (a native conformation) or Form II open circle (resulting from a single-strand break).

### Statistical analysis

Data are reported as means ± SD of plasmid percentage forms. A one-way analysis of variance was performed to verify possible statistical differences. A rigorous statistical post-test (Bonferroni) was chosen to identify the *p*-value (*p* < 0.05 as lesser significant level) and to compare each treated group with the control group (0.9% NaCl). InStat Graphpad software was used to perform statistical analyses (GraphPad InStat version 3.00 for Windows 95, GraphPad Software, San Diego, California, USA).

## Results

Figure 1 shows the survival fractions of *E. coli* AB1157 cultures treated with SnCl<sub>2</sub> in the presence or absence of PEMF, sonic waves, radiofrequency and IPL. The data show no alteration in the survival fraction. Moreover, no protective effect from the physical agents against SnCl<sub>2</sub> action in *E. coli* cultures was found.

The electrophoretic profile of pBSK plasmid DNA under different experimental conditions is shown in Figure 2. In Lane 1, the plasmid DNA alone is found mostly as a supercoil form (Form I). Lane 2 shows the efficient cleavage of the SnCl<sub>2</sub>-induced plasmid DNA, illustrated by the formation of the open circular form (Form II). Lanes 3 to 5 show the electrophoretic profiles of plasmid DNA submitted to physical agents, suggesting no modifications in plasmid topology when compared with the control (Lane 1). Lanes 6 to 8 show that the physical agents did not alter the SnCl<sub>2</sub> action on the electrophoretic mobility of the plasmid DNA.

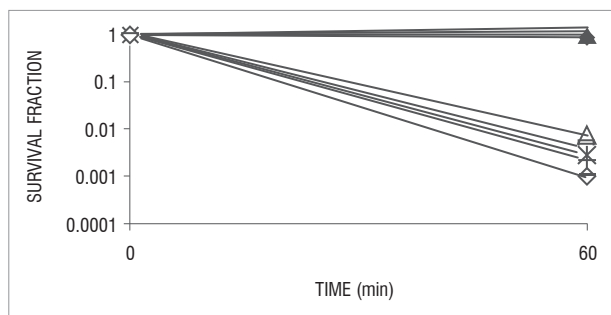


Figure 1: Effects of pulsed electromagnetic fields (PEMF north and south), sonic waves (SW), radiofrequency (ECT) and intense pulsed light (IPL) on the survival fraction and inactivation induced by stannous chloride (SnCl<sub>2</sub>) in *E. coli* AB1157. As a negative control, bacterial cells were incubated with NaCl (0.9%, 60 min) and as a positive control, bacterial cells were incubated with SnCl<sub>2</sub> (25 µg/mL, 60 min); controls were not exposed to the physical agents.

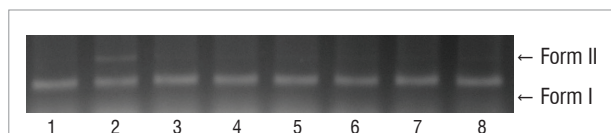


Figure 2: Photograph of agarose gel showing Form I (supercoiled DNA) and Form II (open circle DNA). Samples of pBSK plasmid were exposed to pulsed electromagnetic fields (PEMF north and south), sonic waves (SW), radiofrequency (ECT) and intense pulsed light (IPL). A 0.8% agarose gel electrophoresis (8 V/cm) was performed in tris-acetate-EDTA buffer. As a negative control, plasmids were incubated with NaCl (0.9%, 40 min) and as a positive control, plasmids were incubated with SnCl<sub>2</sub> (200 µg/mL, 40 min); controls were not exposed to the physical agents.

Figure 3: Bar chart showing the percentage of plasmid pBSK in Form I (supercoiled DNA) and Form II (open circle DNA) across 8 lanes. The y-axis is Percentual Form (0 to 100). The x-axis is Lane (1 to 8). Lane 1 is the negative control, Lane 2 is the positive control, and Lanes 3-8 show various physical agents. Lane 2 shows a high percentage of Form II (approximately 25%), while all other lanes show a high percentage of Form I (approximately 90%).

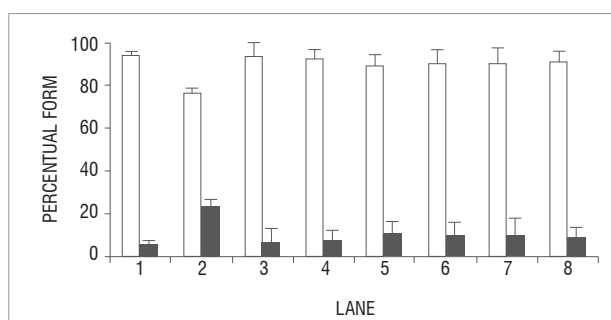


Figure 3: Percentage of plasmid pBSK in Form I (supercoiled DNA) and Form II (open circle DNA) exposed to pulsed electromagnetic fields (PEMF north and south), sonic waves (SW), radiofrequency (ECT) and intense pulsed light (IPL). A 0.8% agarose gel electrophoresis (8 V/cm) was performed in tris-acetate-EDTA buffer. Unexposed plasmids were used as a negative control and plasmids incubated with SnCl<sub>2</sub> (200 µg/mL, 40 min) were used as a positive control. Semiquantitative densitometric measurements of Form I and Form II were made using the Gimp computer program.

## Discussion

There is little information about the biological effects of sonic and radiofrequency waves, low intensity magnetic fields and IPL that have been used in therapeutic practice.<sup>18</sup> Doubts related to safety persist owing to the lack of a scientific explanation for their mechanisms of action, as well as for the characterisation of parameters that may or may not have a harmful effect. The correct use of a physical agent is important, both for protecting public health and for ensuring exposure levels that result in the desired biological effects.<sup>19</sup>

The success of treating ulcers using PEMF appears to be as a result of fibroblast production stimulation and bactericide effects.<sup>20</sup> Capponi and Ronzio<sup>4</sup> suggest that PEMF is contraindicated in the presence of fungal and viral infections, because of its cell proliferation effects. Magnetostatic bacteria are extremely affected by PEMF; however, our data indicate that PEMF did not alter *E. coli* cell survival (Figure 1).

Studies using PEMP at a low frequency have reported nerve repair,<sup>21,22</sup> increased osteogenesis,<sup>5</sup> reduced hyperthrombocythemia and hyperfibrinogenemia,<sup>23</sup> improvement in tropical ulcers<sup>24</sup> and DNA synthesis.<sup>4</sup> Kulishova et al.<sup>25</sup> showed the efficacy of general magnetotherapy in conservative therapy of uterine myoma in women of reproductive age.

Our data also indicate that PEMF could not induce DNA strand breaks, at least when the technique involving DNA electrophoresis mobility in agarose gel was used (Figures 2 and 3). However, epidemiological data have suggested that PEMF may be a risk factor for breast cancer in humans.<sup>26</sup>

Radiofrequency could have both thermal and non-thermal effects. Ubeda<sup>27</sup> reported cytotoxic effects in neuroblastoma and hepatocarcinoma cells in the non-thermal modality that controls the cancer's development. Despite Ubeda's findings, we found no effects of radiofrequency on *E. coli* cultures (Figure 1) and plasmid DNA (Figures 2 and 3). Ley-Valle<sup>28</sup> described undesirable effects on the central nervous system when using 2-MHz radiofrequency.

Positive effects of sonic waves, that is increased collagen synthesis, have been described when used to treat scarring in humans.<sup>4</sup> However, harmful effects were reported by Lennart<sup>29</sup> who used ultrasonic (1 MHz) and high-intensity (>30 W/cm<sup>2</sup>) waves. Araújo et al.<sup>30</sup> showed that 3 MHz, 3 W/cm<sup>2</sup> ultrasound waves, in stationary and continuous application, stimulated venous thromboembolism and increased the number of lymphocytes. It has been described that these waves affect protozoans, inactivate viruses, destroy red blood cells and bacteria and hinder fungal multiplication.<sup>28</sup> However, in this study, using 3.3-KHz sonic waves, no effects on bacterial cultures (Figure 1) or plasmid DNA (Figures 2 and 3) were found. In addition, our data suggest that sonic waves could not protect *E. coli* cells against the cytotoxic effect of SnCl<sub>2</sub> or increase the action of this reducing agent.

Intense pulsed light systems are high-intensity light sources that emit polychromatic and non-coherent light, allowing great variability in the selection of individual aesthetic skin treatments<sup>31</sup> such as facial rejuvenation,<sup>32</sup> or for the treatment of skin diseases such as erythrodermia.<sup>33</sup> Isaac et al.<sup>12</sup> and Perez Rivera et al.<sup>10</sup> described the results of treating stains and benign vascular lesions (haemangiomas). Patients treated with IPL at 420–950 nm showed a return to baseline of their facial acne.<sup>34</sup> Acne pathogenesis is believed to involve sebaceous follicular hyperplasia, hyperkeratinisation, *Propionibacterium acne* proliferation, inflammation and immune reactions. It has been suggested that IPL may decrease sebaceous gland size, pilosebaceous inflammation and *Propionibacterium* species populations,<sup>35</sup> but the results obtained in our study, under the test conditions, indicate no effect of IPL on *E. coli* cells (Figure 1) or plasmid DNA (Figures 2 and 3). Furthermore, no protective effect on the *E. coli* culture against the toxic effect of SnCl<sub>2</sub> (Figure 1) was found.

In conclusion, at least under the conditions used in this investigation, as well as for the techniques used, our data suggest that low-frequency pulsed electromagnetic fields, audible sonic waves, radiofrequency

and IPL do not have important biological effects. Moreover, these sources of energy do not modify the cytotoxic and genotoxic effects of SnCl<sub>2</sub> on *E. coli* cells and on plasmid DNA.

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## Authors' contributions

M.B.F. was the project leader; P.F.M., A.S.F. and O.A.R. performed most of the experiments; S.D.S.F. made conceptual contributions; A.S.F. performed the plasmid experiments; and S.D.S.F., M.B.F. and P.F.M. wrote the manuscript.

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