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Experimental setup for magnetic hyperthermia: pilot study

M.E. Cano[a]*, T. Córdova[b], A. Hernández[b], J.C. Estrada[a], P. Knauth[a], Z. López[a], M. Sabanero[c], and M. Sosa[b].
[a]*Centro Universitario de la Ciénega de la Universidad de Guadalajara,*
Av. Universidad 1115, Col. Linda Vista, C.P. 47820, Ocotlán Jalisco México.
**e-mail: eduardo.cano@cuci.udg.mx, meduardo2001@hotmail.com.*
[b]*Departamento de Ingeniería Física – DCI, Universidad de Guanajuato campus León,*
Loma del Bosque 103, Lomas del Campestre, 37150 León, Gto., Mexico.
[c]*Departamento de Biología – DCE, Universidad de Guanajuato campus Guanajuato*
Noria alta s/n, Noria Alta, 36050 Guanajuato, Gto., Mexico.

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In an experimental setup for magnetic stimulation, phantoms were marked with commercially available magnetite powder and placed in the inner induction coil. The inverter circuit and its pulse width modulation control driver circuit (PWM) generated an alternating magnetic field with a frequency of 200 kHz and an amplitude of 10 mT to heat up the phantoms. Water-phantoms (1 ml) could be heated up to 45 °C within 4 to 11 s using 1.0 or 0.6 g/l of magnetite. Heat induced changes in the tertiary and/or secondary structure of trypsin, either by magnetic induction or using a water bath, could only be obtained after heating up the samples for about 20 min, while short time heating (up to 6 min) had any effect. These preliminary results are indicating that magnetic hyperthermia can produce changes in biological systems and $e.g.$ causing necrosis or inducing apoptosis in tumor cells.

Keywords: Hyperthermia; magnetic nanoparticles.

Fue desarrollado un montaje experimental para llevar a cabo la estimulación magnética de muestras preparadas de laboratorio marcadas con magnetita en polvo comercial al ser colocadas en el interior de una bobina inductiva. El circuito inductivo y su controlador de ancho de pulso modulado (PWM) generan un campo magnético alterno de 200 kHz de frecuencia y una amplitud de 10mT para calentar las muestras. Las muestras preparadas de de agua (1 ml) pudieron ser calentados hasta los 45 grados en un tiempo de 4 a 11segundos usando 1.0 o 0.6 g/l de magnetita. El calentamiento produjo cambios en la estructura terciaria y/o secundaria de Trypsina ya sea por inducción magnética o con un baño de agua, sólo después de calentar las muestras durante 20 minutos, mientras que el tiempo de calentamiento a corto plazo (hasta 6 min) no se tuvo ningún efecto. Estos resultados preeliminares son indicadores de que hipertermia magnética puede producir cambios en sistemas biológicos y causar necrosis o inducir apoptosis en células tumorales.

Descriptores: Hipertermia; nanopartículas magnéticas.

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1. Introduction

Magnetic hyperthermia is a relatively new alternative and low invasive for the treatment of cancer [1,2]. The objective of this technique is the necrosis of malignant cells, by induced heating to temperatures of 45 °C, using alternating magnetic fields of remote frequencies from the ionising radiation [3–5]. Functionalised superparamagnetic or ferromagnetic particles [6,7], with a diameter between 10 to 120 nm [8–11], have to be introduced into tumor cells, in order to stimulate them magnetically.

The heating of the magnetic tracer embedded in a living tissue may be due to: the Brownian effect; Eddy currents; fluctuations in the directions of the magnetic moments, crossing the anisotropic barrier of the tracer (Neel relaxation); or to the magnetic energy loss due to magnetic hysteresis loop [12,13]. In each mentioned case are involved important factors, such as: the viscosity of the medium, stimulation frequency and size of the magnetic tracers.

The heating capacity of a magnetic tracer is quantified by determining the specific absorption power rate (SAR). This is the amount of the magnetic energy converted in heat per time and mass. SAR can be expressed as (Eq. 1).

$$SAR = C_s \frac{\Delta T}{\Delta t}, \tag{1}$$

where C_s is the heat capacity of the sample.

The aim of this work is to present the preliminary advances of magnetically induced heating in laboratory phantoms, using a magnetic powder as a tracer, which derived from commercial ferromagnetic magnetite. The magnetic stimulator, developed in our laboratory generates alternating magnetic fields of frequency of around 200 kHz with an amplitude around of 10 mT. Finally, a discussion is provided on the prospects of this work, implementing *in-vitro* experiments on necrosis and apoptosis in cell culture, by using functionalised ferromagnetic or superparamagnetic nanoparticles.

2. Procedure

In Fig. 1A and Fig. 1B is shown a general scheme for an experimental setup for conducting magnetic hyperthermia studies. The inverter circuit and its pulse width modulation control driver circuit (PWM) were fed with a $250W$ DC power supply. The inverter feeds a resonant LC (tank circuit), which

generates an electric current in resonance of about $I = 30A$, which was measured from peak to peak and with a total harmonic distortion (THD) smaller than 1% (measured at the third harmonic after the fundamental frequency according to Eq. (2)).

$$THD = \frac{\sqrt{Y_2^2 + Y_3^2 + \cdots + Y_n^2}}{Y_1}, \tag{2}$$

where $Y_i : i = 2, 3, \ldots, n$ are the harmonics of the fundamental frequency Y_1.

This current is sufficient to generate an alternating magnetic field with a frequency of *200 kHz* and an amplitude of *B = 10 mT*; this is consistent with Eq. (3).

$$B = \frac{\mu_0 IN}{l}, \tag{3}$$

where $\mu_0$is the permeability of the empty space and l the length of the coil. Similar kinds of electronic topology have been studied also by others [14,15].

Due at the high current crossing the induction coil (*L = 6 μH*), it is necessary to cool the coil with water ($2l/s$); therefore the inductor L was made with $N = 10$ turns of copper tube, with an inner diameter of $d = 4$ *cm* and a core of air.

The resonance frequency and current resonance were calculated by using the Eq. (4) and (5).

$$f = \frac{1}{2\pi\sqrt{LC}}, \tag{4}$$

$$i = V_{DC}\sqrt{\frac{C}{L}}. \tag{5}$$

Here $C = 0.1\mu F$ is the capacitance and V_{DC} is the voltage of the power supply.

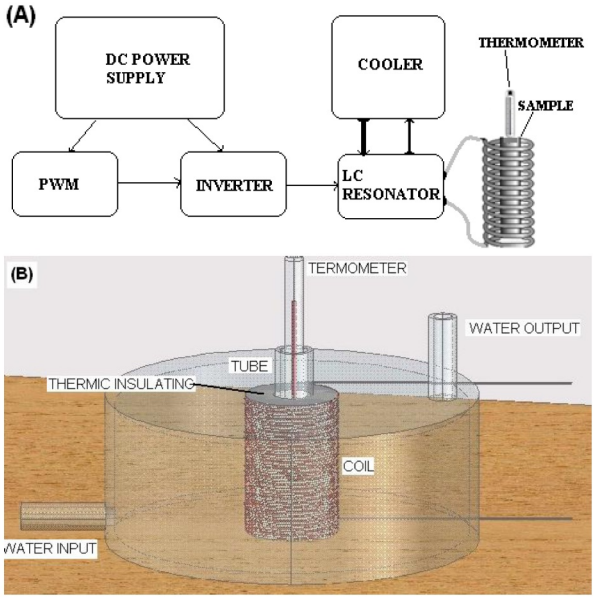

FIGURE 1. Scheme for the general setup of the experiment (A) and of the induction coil with its cooling element (B).

The magnetic stimulator was used to heat four laboratory phantoms: tubes with a diameter of *1.5 cm* containing *1.0 ml* distilled water and four different concentrations of the magnetic tracers (0.4 g, 0.6 g, 0.8 g and 1.0 g, respectively). The tubes were placed in the centre of the induction coil and heated to 45 °C by magnetic induction. At higher concentrations of the magnetic tracers, the phantom could be heated to 45 °C faster (Fig. 2). The temperature was measured with an alcohol thermometer and a digital camera controlled by a PC.

Afterwards the heat induction was examined using biological phantoms: a protein solution (2.5 mg/ml trypsin [Gibco] in HBSS-buffer, pH 7.1). Trypsin is a serine protease found in the digestive system, which is produced in the pancreas as the inactive proenzyme trypsinogen and is then secreted into the duodenum. After limited proteolysis it is converted into the active β-trypsin, which in turn can be converted to α-trypsin by autolysis. Trypsin has a low molecular weight of about 23.5 kDa, a temperature optimum of 37 °C, a pH optimum of 7-9 and is still active in solutions with 0.1% SDS [16,17]. The samples were heated to 45 °C by magnetic induction using 1.0 g/ml magnetic tracers, the positive controls were heated in a water bath and the negative control was not heated. In a first set of experiments a short time scale (2, 4 and 6 min, respectively) and in a second set longer heating periods (18 and 23 min, respectively) were investigated. The influence of the heat on the protein solution, $i.e.$ heat induced changes in the tertiary and/or secondary structure of the trypsin, was revealed by separating the protein by sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) at 12% and staining of the developed gel with Coomassie blue.

3. Results

For the first set with short time heating of the samples, a major protein band of about 25 kDa can be seen in the samples

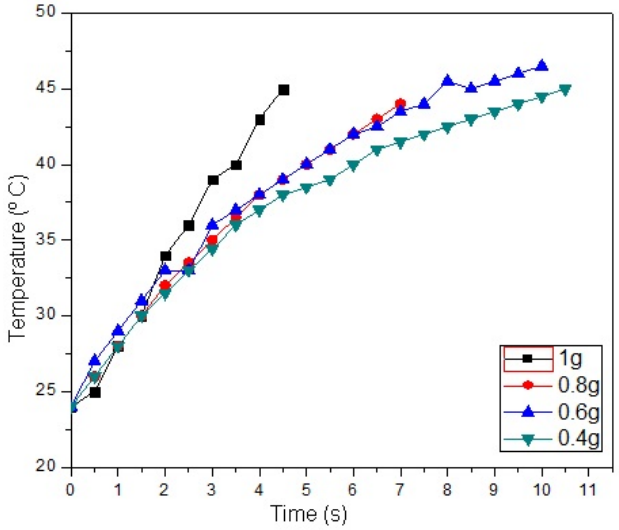

FIGURE 2. Influence of the concentration of magnetic tracers on the time to heat water phantoms to 45 °C.

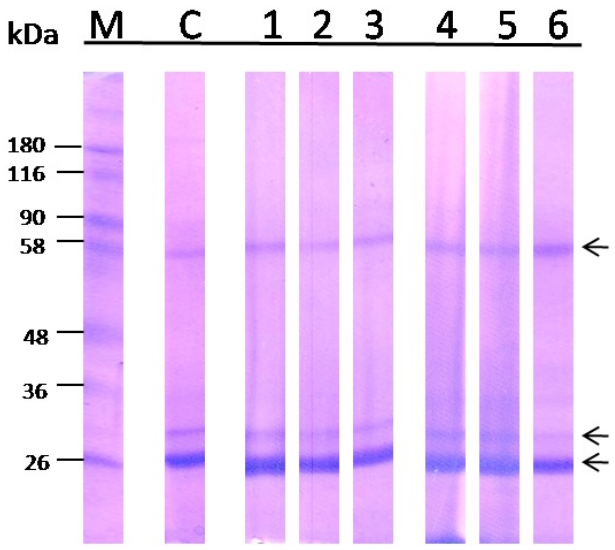

FIGURE 3. SDS-PAGE of heat treated trypsin samples: Lane 1-3 heated by magnetic induction for 2, 4 and 6 min, respectively; lane 4-6 heated in a water bath for the same times (pos. control); lane "C" non heated neg. control. Lane "M" is the size marker. Arrows indicate peptides of about 25, 30 and 55 kDa.

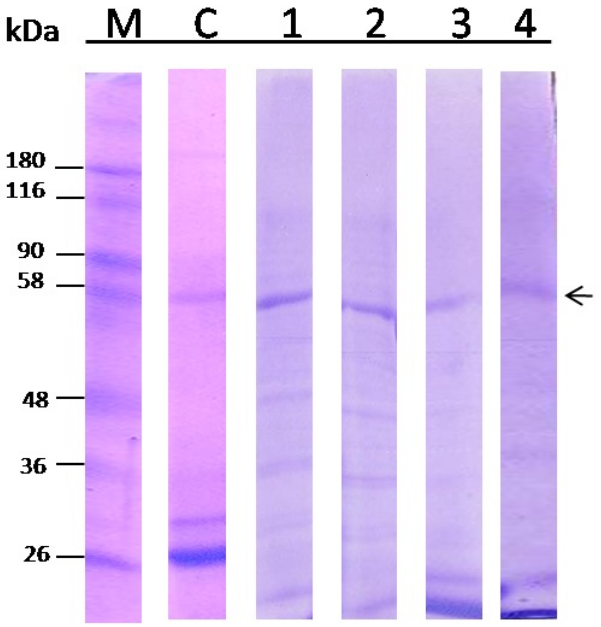

FIGURE 4. SDS-PAGE of heat treated trypsin samples: Lane 1-2 heated by magnetic induction for 18 or 23 min; lane 3-4 heated in a water bath for the same times (pos. control); lane "C" non heated neg. control. Lane "M" is the size marker. Arrow indicates a peptide of about 55 kDa. The peptides of about 25 and 30 kDa, visible in the neg. control, have disappeared in the samples (1-2) and pos. control (3-4).

(Fig. 3, lanes 1-3), in the positive controls (Fig. 3, lanes 5-6) as well as in the negative control (Fig. 3, lane "C") that represents the trypsin (23.5 kDa) itself. The two minor protein bands of about 30 and 55 kDa, respectively, are typical residual peptides, which could not be eliminated during the purification process of the trypsin. With this short time heating, no differences between the samples, the positive control or the negative control could be detected, indicating that by this treatment the protein structure was not destroyed noticeably.

For the second set with the long time heating of the samples, the major protein band of about 25 kDa has disappeared in the samples (Fig. 4, lanes 1-2), in the positive controls (Fig. 4, lanes 3-4) but still is visible in the negative control (Fig. 4, lane "C"). This demonstrates that with heating by magnetic induction for about 20 min a biological sample can be destroyed considerably; in the same manner like heating in a water bath (pos. control).

4. Discussion

These first results suggest that a larger exposure time magnetic hyperthermia is needed for a gradual degradation of the proteins. These alterations may cause necrosis or induce apoptosis in (cancer) cells.

In this paper were presented the progress in the experimental setup for studies of magnetic hyperthermia. In order to extend this type of experiments in biological tissues, interesting in the oncology area, it is necessary to optimise several parameters such as:

Cytotoxicity of the magnetic tracers.

Efficiency in the magnetic marking process.

Optimisation of the magnetic properties and size distribution of the magnetic tracers.

The determination of all these mentioned parameters is the starting point to perform studies related with the apoptosis and necrosis phenomena in cell culture.

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1. M. Johannsen, *et al.*, *Actas Urol Esp.* **31:**(6) (2007) 660.
2. M. Yanase, *et al.*, *Jpn. J. Cancer Res.* **89** (1998) 463.
3. R. Hergt, S. Dutz, R. Müller, and M Zeisberger, *J Phys: Condens Matter* **18** (2006) s2919.
4. A. Jordan, R. Scholz, K. Maier-Hau, *Journal of Magnetism and Magnetic Materials* **225** (2001) 118.
5. C.F. Carobbi, S. Lazzerini, and L.M. Millanta, *IEEE Transactions on instrumentation and measurement* **54:**(4) (2005) 1427.
6. D. Hellstern, *et al.*, *Journal of Nanoscience and Nanotechnology* **6** (2006) 1.
7. C.C. Berry, *J. Phys. D: Appl. Phys.* **42** (2009) 224003.
8. D. Bahadur and J. GIRI, *Sâdhanâ* **28**:(3,4) (2003) 639.
9. Q.A. Pankhurst, N.K. Thanh, S.K. Jones, and J. Dobson, *J. Phys. D: Appl. Phys.* **42** (2009) 224001.
10. A.H. Grande, *Rev. R. Acad. Cienc. Exact. Fís. Nat. (Esp)* **101:**(2) (2007) 321.
11. M. Bettge, J. Chatterjee, and Y. Haik, *BioMagnetic Research and Technology* **2**:4 (2004) 1.
12. A.A. Kuznetsov, O.A. Shlyakhtin, N.A. Brusentsov, O.A. Kuznetsov, *European Cells and Materials* **3**:(2) (2002) 75.
13. G. Nedelcu, *Digest Journal of Nanomaterials and Biostructures* **3**:(3) (2008) 103.
14. D. Kirubakaran S.R. Reddy, *Journal of Industrial Technology* **25**:(2)(2009) 1.
15. A. Weber, E. Carroll, M Frecker, *ABB Switzerland Ltd* **1** (2002) 1.
16. K.A. Walsh, *Trypsinogens and trypsins of various species. Meth. Enzymol.* **19** (1970) 41.
17. B.J. Smith, *Methods of Molecular Biology.* **Vol. 3** (Humana Press, New Jersey 1988) 57.