Studies of Ferrite Based Magnetic Nanoparticle Uptake and Magnetocytolysis Effects on Model Cell Cultures

H.L. Rodriguez, A. Herrera, C. Barrera, C. Rinaldi, and M. Torres-Lugo

*University of Puerto Rico, Mayagüez, PR, USA
hlrodri@gmail.com, crinaldi@uprm.edu, madeline@ece.uprm.edu

ABSTRACT

This work focuses on the examination of the physicochemical interactions of magnetic nanoparticles with various cell lines. The applied field maintained a temperature range between 41°C and 45°C at a frequency of 237 kHz and a magnetic field of 2.8 kA/m. Two cell models (Caco-2 and MCF-7) were employed. Magnetite nanoparticles were synthesized by the coprecipitation and thermodescomposition method. They were decorated with carboxymethyl-dextran (CMDX-mag) and Poly (ethylene glycol) (PEG-mag) polymers. Nanoparticle uptake was found to be affected by nanoparticles functionalization and cell type. CMDX-mag nanoparticles were selected to perform MFH experiments. Viability analysis when MFH was applied indicated a significant decrease in cell viability for a magnetic field exposure of 120 min and a resting time of 24 hours when compared to hypethermia caused by a hot water bath. MFH appears to have a higher effect than hyperthermia with hot water bath on cell viability.

Keywords: nanoparticle, magnetite, carboxymethyldextran, magnetic fluid hyperthermia (MFH)

1 INTRODUCTION

Nanoparticles are of special interest due to their unique chemical and electronic properties and because of their potential in optics, luminescence, electronics, catalysis, solar energy conversion, and optoelectronics [1]. Biomedical applications of such systems include biological cell imaging, photothermal therapeutics, cancer treatment, and oral delivery systems that overcome biological barriers.

Ferrofluids are a novel category of nanoparticles used in biomedical engineering. These are colloidal suspensions of iron oxide magnetic nanoparticles. They have several biological applications including magnetic cell sorting, medical imaging, therapeutics, and magnetocytolysis or magnetic fluid hyperthermia [2]. Such nanoparticles can be coated with polymers like dextran, albumin, organosilanes or methacrylates to prevent agglomeration. This coating also helps with particle biocompatibility.

Hyperthermia is the increase of tissue temperature to 41-46°C. Previous work has shown that this temperature range can be reached using magnetic nanoparticles and the application of an oscillating magnetic field [3] exploiting the opportunity that cancerous cells are more susceptible to heat than normal cells. This treatment is suitable for localized tumors only.

Nanoparticle rotation caused by the magnetic field dissipates energy. This dissipation increases tumor temperature, leading to cell death by hyperthermia, while minimizing heating of the rest of the organism. This is known as magnetic fluid hyperthermia (MFH). Investigators have measured the temperature increase in *in-vivo* experiments and have noticed cell death or tumor remission after application of an AC magnetic field. [3].

This work envisions the understanding of the physicochemical interactions of magnetic nanoparticles with various cell lines and the underlying cell mechanisms. For this purpose, we applied a magnetic field that maintained a temperature range between 41° C and 45° C (hyperthermia limits), varying only the time of application. With two different cell models we investigated the effect of magnetic fluid hyperthermia on cell death and the potential mechanisms.. We also analyzed the importance of magnetic field exposure time on cell death. Magnetic fluid hyperthermia results were compared to hyperthermia analysis caused by hot water bath. Statistical differences on the results indicate that other mechanisms in addition to heat may also contribute.

2 MATERIALS AND METHODS

2.1 Materials

Iron (II) chloride tetrahydrate ($FeCl_2 . 4H_2O$) 99%, iron (III) chloride hexahydrate ($FeCl_3 . 6H_2O$) 97%, Carboxymethyl Dextran Sodium Salt, ammonium hydroxide (NH_4OH) 29% v/v, epichlorohydrin 99%, and Dulbecco's Modified Eagle's Medium (DMEM were purchased from Sigma Aldrich. CaCo-2 cells, MCF-7 cells and were purchased from American Type Culture Collection.

2.2 Magnetic nanoparticles synthesis

Nanoparticle synthesis was performed as previously reported described [4-6].

2.3 Nanoparticle Suspension

Nanoparticles were autoclaved for 60 minutes at a temperature of 121°C and 18 psi. Characterization analysis of the particles was performed after the autoclave process to verify particle integrity. No agglomeration and no loss of particle coating was detected. Particles were suspended in supplemented Dulbecco's Modified Eagle's Medium with phenol red containing 10% of Fetal Bovine Serum. Final particle concentration was 10 mg/mL (3.0 mg magnetite/mL).

2.4 Cellular Nanoparticles Uptake

Cells at a concentration of 50,000 cells/cm^2 were seeded in sterile 6 well plates (3502, Falcon, Corning, NY) and cultivated for one week in supplemented DMEM with phenol red at 37° C and 5% of CO_2. After one week, cells were placed in contact with a solution of 0.30 mg magnetite/mL diluted in DMEM for a desired period of time. Nanoparticle suspension was then removed and cells were rinsed with ice-cold BRS to remove excess particles. The cell monolayer was trypsinized for 20 minutes at 37°C. Detached cells were counted and centrifuged. The resulting pellet was solubilized with 0.5% Triton-X 100 (Sigma, St. Louis, MO) in BRS for 30 minutes at 37°C. Finally BRS was added to a final volume of 1 mL. The samples were analyzed using an Inductively Couple Plasma Mass Spectroscopy (7500, Agilent, CA).

2.5 Hyperthermia with Hot Water Bath

Cells were cultivated on 75 cm^2 cell culture flasks (canted neck) (Costar, Corning, NY) using Dulbecco's Modified Eagle Medium (DMEM) (Sigma, St. Louis, MO) containing 10% fetal bovine serum (Invitrogen, Carlsbad, CA), 1% nonessential amino acids (Invitrogen, Carlsbad, CA) 100 units/mL of penicillin (Sigma, St. Louis, MO), and 100 μg/mL of streptomycin (Sigma, St. Louis, MO). Cells were maintained in a controlled atmosphere at 37 ºC, 95% relative humidity, and 5% CO_2 during one week. Cells were detached from the culturing flask by trypsinization, resuspended in culture media, and counted. A concentration of 3.0 E 6 cells were placed on a 20 mL test tube and brought to a final volume of 10 mL with DMEM. The test tube was then placed on a hot water bath for the predetermined time at a constant temperature (41ºC or 45ºC). After hyperthermia treatment cell viability and apoptosis was analyzed.

2.6 Magnetic Fluid Hyperthermia

Cells were treated as described in section 2.5. A concentration of 3.0 E 6 cell was seeded on a 20 mL test tube and brought to a final volume of 15 mL with DMEM (negative control) or with a concentration of 2 mg/mL of magnetite carboxymethyl-dextran nanoparticle solution. The test tube was then collocated in the coil of the heat induction equipment. The solution was purged with air to maintain cell suspension during the application of the magnetic field. . The field was applied for a period of 1 hour or 2 hours with a frequency of 237 kHz and a magnetic field of 2.8 kA/m. It was also performed for two times with 1 hour of application with an interval of 4 hours between each treatment. After the magnetic field treatment the cell viability and apoptosis was analyzed.

The effect of particle internalization in cell viability after a magnetic field application was also studied. The cells were seeded as described herein but they were in contact with a magnetite carboxymethyl-dextran nanoparticle suspension of 1 mg/mL during cell seeding. The magnetic fluid hyperthermia protocol was performed as described.

Three different controls were performed, suspended cells incubated with DMEM, suspended cell incubated with CMDX-mag nanoparticle solution, and cell suspended on DMEM exposed to a magnetic field without nanoparticle suspension.

2.7 Cell viability and Apoptosis

Cell viability and apoptosis analysis was performed immediately or 24 hours after the magnetic field application. Cell viability was performed using the Trypan Blue exclusion method. Cells were counted before and after the magnetic field application. The results were presented as a normalized ratio with the negative control. Apoptosis was analyzed using ApoPercentage (Accuratechemical, Westbury, NY) assay. Cells were put in contact with the assay and counted. Results were presented as the ratio of the apoptotic cells normalized with the number of total cells counted.

3 RESULTS

The kinetics of nanoparticle uptake was analyzed at confluency for a contact period of 24 and 48 hours on Caco 2 and MCF 7 cell models. Figure 1 shows that carboxymethyldextran-magnetite nanoparticles (CMDX-mag) were incorporated approximately 0.5-0.6 pg magnetite/cell either at 24 or 48 hours in contact. Such results may indicate that saturation occurred after 24 hours. CMDX-mag on MCF-7 had an uptake of 0.1 and 0.3 pg magnetite/cell for 24 and 48 hours, respectively. MCF-7 showed a distinct uptake behavior which may indicate a slower particle internalization process. Poly(ethylene glycol) magnetite (PEG-mag) demonstrated a reduced internalization on Caco-2 cells when compared with CMDX-mag at 24 hours of contact (0.05 pg magnetite/cell) while for 48 hours in contact the internalization was 0.4 pg magnetite/cell. This may suggest that PEG-mag nanoparticles may be interacting by other mechanisms. MCF-7 showed the same uptake behavior of PEG-mag nanoparticles when compared to CMDX-mag nanoparticles.

Nanoparticle uptake was also examined during cell seeding and growth (see Figure 2). CMDX-mag uptake on Caco-2 cells did not show difference in uptake when

compared to the 24 hours in contact. This suggests fast uptake saturation on this cell model. However, the uptake of CMDX-mag increased to 0.45 pg magnetite/cell in MCF-7 indicating the presence of a slower process. PEG-mag uptake in Caco-2 did not change for the 1 week contact period or the 48 hours in contact (0.5 pg magnetite/cell). On the other hand, PEG-mag on MCF-7 showed an increased in nanoparticle uptake to a value of 0.9 pg magnetite/cell, being the highest internalization obtained for these two particles. The aforementioned results indicated that nanoparticles uptake is affected by particle functionalization and cell type.

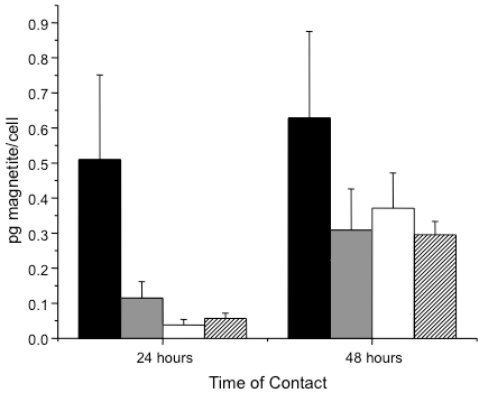

Figure 1. Uptake analysis with confluent cell monolayer: CMDX-mag on Caco-2 cell model CMDX-mag on MCF-7 cell model PEG-mag on Caco-2 cell model PEG-mag on MCF-7 cell model

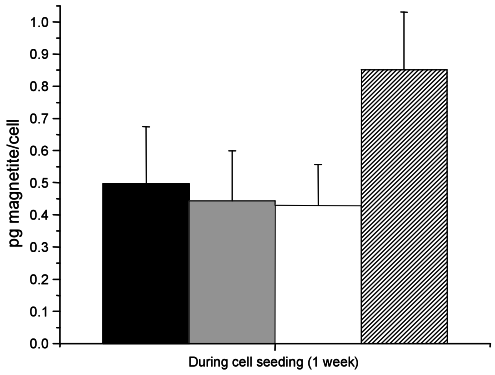

Figure 2. Uptake analysis during cell seeding (1 week): CMDX-mag on Caco 2 cell model CMDC-mag on MCF 7 cell model PEG-mag on Caco 2 cell model PEG-mag on MCF 7 cell model

Hyperthermia analyses were performed on a hot water bath at two different temperatures 41°C and 45°C for a period of 2 hours. Cell viability was analyzed immediately after the 2 hours application or 24 hours after the hyperthermia treatment. Viability analysis after the described treatments (see Fig. 3) resulted on a reduction of approximately 10% when compared to the negative control. All treatments resulted in the same viability reduction.

This analysis was also performed on MCF-7 cells with the same application employed to Caco 2 cells, but only at 45°C. The viability of the MCF 7 cell (Fig. 3) was reduced approximately by 10-20% when compared to negative control in all cases.

Apoptosis analysis was also performed (data not shown). Results demonstrated an increase in apoptosis when samples were compared to control in both cell models. Such results indicated that the apoptotic death is slow and such experiments will be repeated at longer resting times to see the onset of death from those apoptotic cells observed at 24h after treatment.

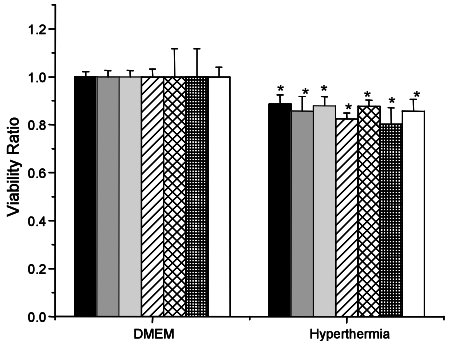

Fig. 3. Viability analysis after hyperthermia caused with a hot water bath: Caco 2 viability measured 24 hours after 2 hours at 41C Caco 2 viability after 2 hours at 45C Caco 2 viability measured 24 hours after 2 hours at 45C Caco 2 viability measured 24 hours after two intervals of 1 hour at 45C MCF 7 viability after 2 hours at 45C MCF 7 viability measured 24 hours after 2 hours at 45C MCF 7 viability measured 24 hours after two intervals of 1 hour at 45C (*sample are different from control but statistically the same between them with $p>0.05$)

Magnetocytolysis or magnetic fluid hyperthermia was performed only varying the time of magnetic field application. The temperature for this treatment was monitored all the time during the magnetic field application with an infrared camera and an alcohol thermometer. The temperature measured during the experiment was in between 41-45°C.

Results using the Caco2 cell line indicated that the highest effect was observed for a contact period of 2 hours after a resting time of 24 hours regardless if the application process was divided in two one hour intervals or if the nanoparticles were allowed to be internalized for a period of one week during growth. These results suggest that the internalization of these particles were not apparently enough to cause a higher effect on cell viability during the application of a magnetic field and a total dose is necessary regardless of the application process. However, when compared to hyperthermia with hot water, a significant reduction was observed when MFH was applied. This may

suggest a possible mechanical effect from nanoparticle rotation.

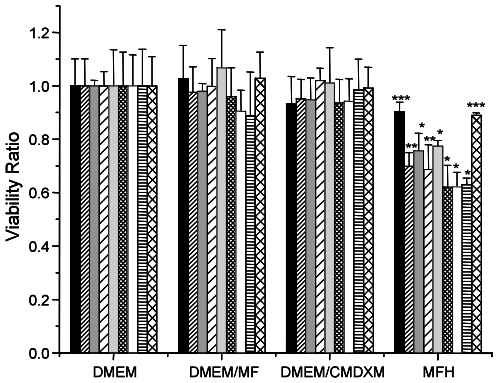

Figure 4. Viability analysis on Caco 2 cells after magnetic fluid hyperthermia (MFH): 1 hours MFH with nanoparticle suspension Measured 24 hours after 1 hour MFH with nanoparticle suspension 2 hours MFH with nanoparticle suspension Measured 24 hours after 2 hours MFH with nanoparticle suspension 2 hours of MFH with nanoparticle suspension after cells were 1 week in contact with nanoparticles Measured 24 hours after 2 hours of MFH with nanoparticle suspension after cells were 1 week in contact with nanoparticles Measured 24 hours after two intervals of 1 hour MFH with nanoparticle suspension Measured 24 hours after two intervals of 1 hour MFH with nanoparticle suspension after cells were 1 week in contact with nanoparticles Measured 24 hours after 2 hours MFH without nanoparticle suspension after cells were 1 week in contact with nanoparticles (*** Samples statistically the same compared to control with p>0.05, **Samples different form control but statistically the same between them with p>0.05 and * Samples different form control but statistically the same between them with p>0.05)

Magnetic fluid hyperthermia was also analyzed on the MCF-7 cell line (Figure 5). Cell viability was measured 24 hours after treatment. When compared to the Caco-2 cell line the viability of the MCF-7 was more profoundly affected.

Also apoptosis analyses were performed (data not shown). Results indicated an increase on apoptosis in all cases and in both cell lines when compared to controls. Studies will be performed at longer resting times to see the effects of the apoptosis process.

CONCLUSION

CMDX-mag nanoparticles appeared to reach saturation on Caco-2 cells after 24 hour of contact, while the uptake on MCF-7 was slower. PEG-mag nanoparticles had a higher uptake on MCF-7 cells suggesting a different internalization mechanism.

Magnetic fluid hyperthermia affected cell viability when a magnetic field was applied for a period of 2 hours. Neither nanoparticle internalization nor the application of the magnetic field on two different intervals produced a higher effect when compared to 2 hours of magnetic field application. Magnetic fluid hyperthermia when compared with hyperthermia with hot water bath suggested that cell viability was affected by a potential mechanical effect due to nanoparticle rotation during magnetic field application.

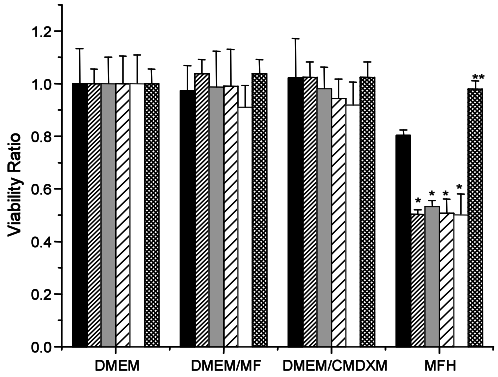

Figure 5. Viability analysis on MCF 7 cells after magnetic fluid hyperthermia (MFH): 2 hours MFH with nanoparticle suspension Measured 24 hours after 2 hour MFH with nanoparticle suspension Measured 24 hours after two intervals of 1 hour MFH with nanoparticle suspension Measured 24 hours after 2 hours of MFH with nanoparticle suspension after cells were 1 week in contact with nanoparticles Measured 24 hours after two intervals of 1 hour MFH with nanoparticle suspension after cells were 1 week in contact with nanoparticles Measured 24 hours after 2 hours MFH without nanoparticle suspension after cells were 1 week in contact with nanoparticles (** Samples statistically the same compared to control with p>0.05, *Samples different form control but statistically the same between them with p>0.05)

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