

Hadron Therapy, magnetic nanoparticles and Hyperthermia: a promising combined tool for pancreatic cancer treatment

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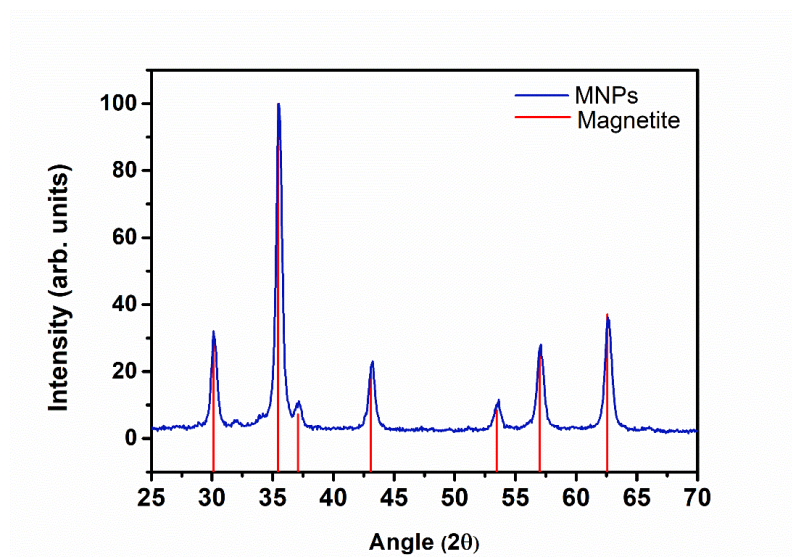


Figure S1. XRD patterns, compared to the reference pattern (red vertical bars) of magnetite (JCPDS 19-0629).

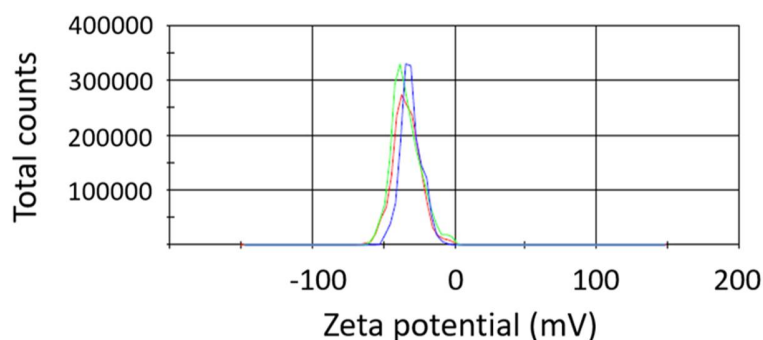


Figure S2. Zeta potential measurements at pH 7.4. The green, blue and red lines represent data obtained from three different measurements. The zeta potential measurements show that the surface charge of the MNPs is negative, ($Z_{\text{pot}} = -30.5 \pm 7.5$ mV at pH 7.4). The negative surface charge is attributed to the carboxyl groups of the DMSA molecules and the high zeta potential value justifies the stability of the sample in water.

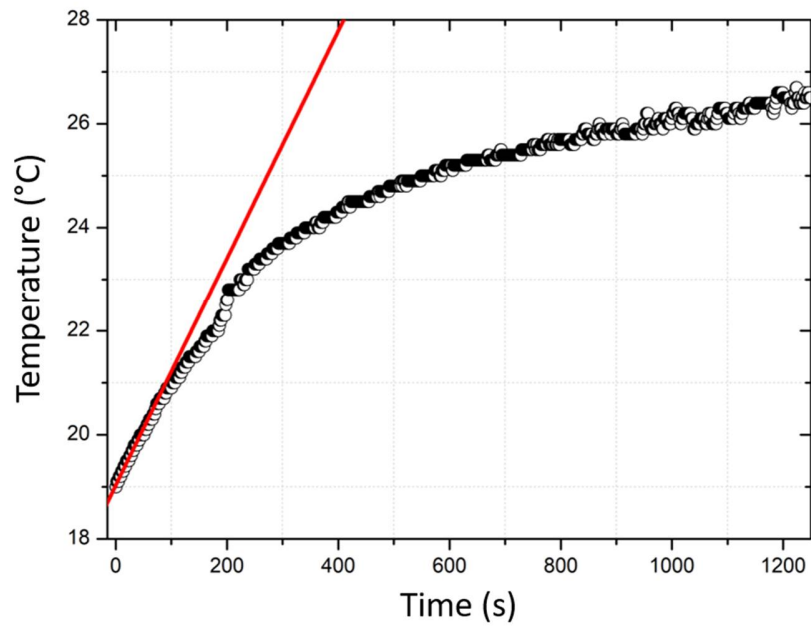


Figure S3. Temperature kinetics (circles) of water suspensions of MNPs as a function of application time interval (Δt) of the alternating magnetic field (amplitude $\mu_0 H = 19.5$ mTesla and frequency $f = 109.8$ kHz). The red line is a fit to the temperature vs. time curve for $\Delta t \rightarrow 0$ (red lines): the SAR was estimated by the so-called initial-slope method, evaluating the rate of temperature increase (from the initial temperature T_0) by a linear fit on a short time range just after the application of the field. At short times, the effect of thermal dissipation can be neglected, and the thermal inertia of the system is taken into account by starting the fit a few seconds (5–20 s) after the application of $\mu_0 H$. The $\Delta T / \Delta t$ ratio for $t \rightarrow 0$ was measured directly from the curve.

Concentration $\mu\text{g/mL}$	Uptake	Survival	σ_{survival}
0	24 h	1.00	0.10
	48 h	1.00	0.10
50	24 h	0.42	0.04
	48 h	0.40	0.04
100	24 h	0.38	0.04
	48 h	0.38	0.04

Table S1. Results of experiments performed to determine the optimal time of uptake and the concentration of MNPs in the cell culture medium are shown. As it can be observed in Table S1, the cells survival obtained for the samples with uptake of the nanoparticles for 24 hours and for 48 hours are almost the same. Moreover, the concentration of 50 $\mu\text{g/mL}$ seems to cause the same cytotoxicity of the concentration 100 $\mu\text{g/mL}$.

Concentration $\mu\text{g/mL}$	Uptake	Survival	σ_{survival}
0	24 h	1.00	0.10
10	24 h	0.88	0.09
25	24 h	0.68	0.07
50	24 h	0.41	0.04
100	24 h	0.38	0.04

Table S2. Results of experiments performed to determine the optimal concentration of MNPs in the cell culture medium. As it can be observed in Table S2, the cell survival (after 15 days from the uptake process) drops increasing the MNPs concentration, reaching a plateau for $c > 50 \mu\text{g/mL}$. Indeed, the concentration of 50 $\mu\text{g/mL}$ and of 100 $\mu\text{g/mL}$ seems to cause the same cytotoxicity effects, probably due to an uptake upper limit due to the small cells internal volume. Finally, we used $c=50 \mu\text{g/mL}$ because with lower c the amount of uptaken MNPs is too low to allow a magnetic hyperthermia treatment, and for $c > 50 \mu\text{g/mL}$ MFH efficacy does not increase.

Sample	G1 phase	S Phase	G2/M Phase	Apoptosi
Control	59.83%	25.4%	13.77%	/
MNPs – 50 µg/ml	57.11%	21.54%	13.25%	8%
MNPs – 100 µg/ml	56.9%	21.43%	13.13%	8.5%

Table S3. Cell cycle analysis of BxPC3 cells measured by flow cytometry after 48 h of treatment with MNP (50 and 100 µg/mL)

Cell cycle distribution was measured after 48 h of incubation with MNPs at concentration of 50 and 100 µg/mL. For cytofluorimetric analysis the cell samples were treated with Ribonuclease A (100 µg/mL—Sigma Aldrich) for 30' at 37 °C and then stained with propidium iodide (50 µg/mL—Sigma Aldrich) for about 12–14 hours. The flow cytometer BD ACCURI C6 was used for the experiments and at least 20,000 cells were counted at each point. The proportion of cells at different phases was gated and calculated using the ModFit Lt software.

There were no significant variations in the values of the different cell phases for all samples, treated with MNPs for 48 hrs and not treated (control). Similar results have been found in several works such as: Ma et al.¹ and Calero et al.², who conclude that MNPs incorporated into cells does not alter the cell cycle. Note instead a certain percentage of apoptotic cells in the samples were treated with MNPs due to the cytotoxicity of the nanoparticles.

Experiment	Cellular Uptake pg _(Fe) /cell
Experiment 1	16.6
Experiment 2	25.6
Experiment 3	17.9

Table S4. Mean cellular uptake (results of ICP measurements).

Dissolution experiments by the radiotracing method.

A dissolution test on Fe₃O₄ nanoparticles (Fe₃O₄-MNPs) in the culture medium (RPMI 1640 media) supplemented with 10% fetal bovine and gentamicin (50 mg/mL) was carried out by the radiotracing method.

Radiolabeled [⁵⁹Fe] ⁵⁹Fe₃O₄-MNPs were prepared at the Triga Mark II Nuclear Reactor of the LENA Laboratory (University of Pavia). The radioactivity measurements were carried out by X-ray spectrometry using a high purity germanium (HPGe) detector (EG&G Ortec, 15% relative efficiency, FWHM = 2.2 keV at 1.33 MeV). Within the experimental errors, the concentration of the irradiated Fe₃O₄-MNPs was in good agreement with the expected nominal one. The ⁵⁹Fe₃O₄-MNPs were resuspended in 10 mL of Millipore H₂O and submitted to the dissolution test by incubating the MNPs in the culture medium at a final concentration of 150 µg mL⁻¹ for 24, 48 and 72 h at 37 °C.³ After incubation the mixtures were centrifuged at 2000g for 10 min and the fraction of ⁵⁹Fe in the supernatants (“ionic Fe”) measured by radioactive counting. The kinetic of the ⁵⁹Fe released by in culture medium (Figure S4) shows concentrations of “ionic Fe” ranging from 1.25% to 3.3% of MNPs mass, suggesting that under our experimental conditions only a small dissolution of ⁵⁹Fe₃O₄-MNPs in culture media occurred.

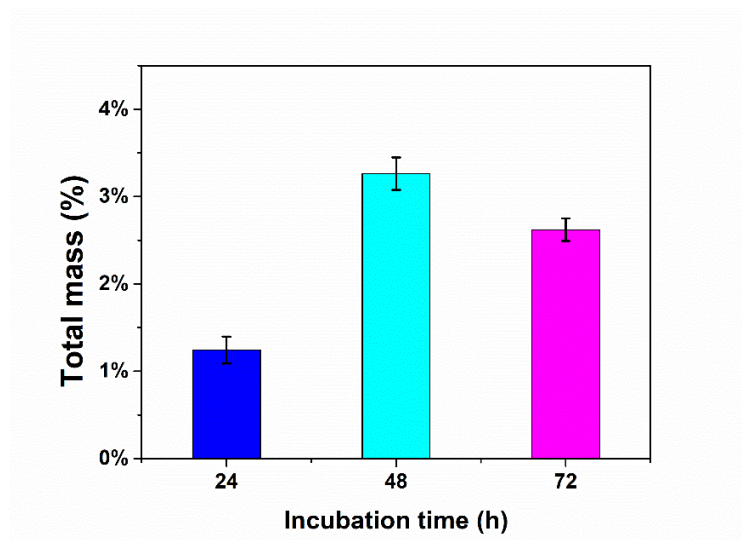


Figure S4. Timed dissolution of $^{59}\text{Fe}_3\text{O}_4$ -MNPs in culture medium. Data are expressed as % of the initial mass of MNPs. Means of three measurements. Bars represent standard errors.

References

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- [2] M. Calero, M. Chiappi, A. Lazaro-Carrillo, M. J. Rodríguez, F. J. Chichón, K. Crosbie-Staunton, A. Prina-Mello, Y. Volkov, A. Villanueva, J. L. Carrascosa, *J. Nanobiotechnology* **2015**, 13, 1.
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