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An iron-oxide nanoparticle with therapeutic capability in Magnetic Fluid Hyperthermia and diagnostic capability in MRI and MPI

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Abstract

M55 belongs to a class of innovative nanomaterials, constituted by doped ferrite, with potential application in tumor therapy, as agents for Magnetic Fluid Hyperthermia (MFH), and in diagnosis, as contrast agents for MRI and MPI. Interestingly, such nanomaterials are characterized by a self-limiting temperature that can be modulated by adjusting the composition of the nanomaterial itself. M55 was coated with a double shell of citrate and glucose. We demonstrated that it has good capability as contrast agent for MRI and MPI. Moreover we tested its biocompatibility in a triple negative human breast cancer line and its efficacy as MFH agent in the same cell line. This agent was highly efficient in decreasing cell viability after two MFH treatments. Finally, we demonstrated that it can be useful to label cells for cell tracking in MPI. Although preliminary, these results are encouraging and push toward in vivo tests of such material.

I. Introduction

M55 belongs to a class of innovative nanomaterials, constituted by doped ferrite, potentially usable as magnetic fluids in Magnetic Fluid Hyperthermia (MFH) that show self-regulating temperature [1, 2]. The self-regulating temperature consists of a stable temperature increase of the magnetic nanomaterial when exposed to alternating magnetic field (AMF) up to a maximum value. This effect provides an intrinsic control for induction heating of relevant interest for in vivo applications in tumor therapy. After coating with a biocompatible citrate and glucose double shell, M55 has been characterized as a bimodal contrast agent in MRI and MPI, as therapeutic agent for MFH in solution and in cell cultures and also as labeling agent for cellular imaging in MPI. Recent studies have reported an innovative application of MPI in MFH. Indeed, by exploiting the Field Free Region (FFR)

of MPI, it is possible to selectively excite only NPs located within such FFR, avoiding unwanted heating of healthy tissues [3,4]. Considering such innovative application, M55 has been investigated as MFH agents by using standard hyperthermia apparatus as well as a MPI imager. Experiments were performed in a triple negative human breast cell line (MDA-MB-231). A recent review reported that breast cancer is the second most commonly diagnosed malignancy, accounting for more than 11.6% of female cancers [5]. Current therapeutic approaches to breast cancer include surgery followed by chemotherapy/radiotherapy an invasive and debilitating approach. Application of MFH to breast cancer could be performed in outpatient therefore avoiding invasive surgery. Our results, although preliminary, are encouraging and push toward in vivo tests of this material.

II. Material and methods

M55 is constituted by a doped ferrite core, synthesized by MBN Nanomaterialia S.p.A (Treviso, Italy) using a proprietary mechano-chemical process [2]. The doped ferrite core was coated with citrate moieties (using Trisodium Citrate dihydrate 99% Alfa Aesar as reagent) and then with glucose (using D-(+)-Glucose SimaUltra 99.5% Sigma as reagent) in order to make it soluble and biocompatible. The synthesis of doped ferrite core and citrate-glucose coating was described previously [1]. Hereafter, the citrate and glucose capped NPs will be referred to as G-M55. Composition and morphology of G-M55 were characterized by X-Ray Powder Diffractometry, TEM, and IR Spectroscopy. DLS was applied to measure the hydrodynamic radius and Z-potential.

The efficiency of G-M55 as multimodal contrast agent was assessed in MPI by using the RELAX module (Magnetic Insight Inc., Alameda, California, USA) and in MRI by using a 7T Bruker Biospec System.

Toxicity of G-M55 was determined by Standard MTT test after incubation with MDA-MB-231 cells (with and without MFH treatment).

SAR was measured using a Nanotherics MagneTherm system (Warrington, United Kingdom) with G-M55 samples diluted in water. The temperature was measured using a multichannel thermometer placed inside the samples. Magnetic field intensity and frequency were 8.75 kA/m and 473.1 kHz respectively. The SAR was also measured by using the HYPER module of the Magnetic Particle Imager Momentum Scanner (Magnetic Insight Inc., Alameda, California, USA.). G-M55 was tested also as a labeling agent for cell tracking in MPI. MDA-MB-231 cells were incubated with 1.0 mg Fe of G-M55 for 24 hours, washed 3 times with 5.0 ml of phosphate buffered saline solution (PBS). Once collected and tossed the supernatant, the pellet was resuspended into 1.0 ml of PBS in order to be imaged in MPI.

Table 1: MPI characterization of M55 and G-M55 in comparison with Vivotrax. Normalized Signal Efficiency is referred to Signal per ng Fe.

NPs	Normalized Signal Efficiency	FWHM (mT)	Approx Resolution (mm)
M55 (isopropyl)	1.8	7.5	0.7
G-M55 (water)	1.3	16.4	1.5
Vivotrax (water)	16.4	11.8	1.1

III. Results and discussion

The Z-potential and hydrodynamic size of G-M55 were investigated through an entire week and average values of -32.1 ± 8.2 mV mV and 113±30 nm were measured, respectively. TEM images revealed that M55 nanoparticles have a large size dispersion with sizes ranging between 10 and 150 nm. FTIR spectroscopy clearly detected the presence of glucose molecules on the surface.

To define the ability of G-M55 to serve as multimodal contrast agent, we assessed the MPI signal efficiency and the MRI transversal relaxation rate. MPI Signal efficiency measures the amount of MPI signal per mass of iron. So higher signal efficiency results in improved signal to noise ratio (SNR) and lower detection limits. In MPI, G-M55 was compared to M55 (naked) and Ferucarbotran (Vivotrax); results are reported in Table 1.

Data reported in Table 1 show that G-M55 and M55 have signal efficiency substantially lower than Vivotrax, but the resolution, computed from FWHM, is comparable to Vivotrax. The resolution, defined as the distance for 2 objects separated at 50%, was estimated according to the following relationship: Resolution (mm) = FWHM (mT)/Gradient (T/m).

Overall, these observations led us to define G-M55 (and M55) a good contrast agent for MPI even with lower signal efficiency than commercially available VivoTrax. Further refinements in the synthesis procedure will be needed to sharpen and intensify the signal efficiency of M55 and its coated version G-M55. The transversal relaxivity in MRI amounted to about 53 $mM^{-1}s^{-1}$, not far from the values of commercial contrast agents.

Before using NPs as hyperthermia mediators, it is crucial to evaluate their biocompatibility with in vitro cytotoxicity assay. After 2, 24 and 48h of incubation with G-M55, the viability of MDA-MB-231 cells was evaluated by using MTT assay. Data reported in Figure 1 show that G-M55 is safe up to 150 μg/mL for long incubation time (48 h).

The specific absorption rate (SAR) of G-M55 was estimated from the thermograms reported in Figure 2 a,b. We should mention that such thermograms were acquired in air thermostated at 36°C. The SAR estimate amounted to 11.1±1.0 W/g and 9.5±1.4 W/g, when evaluated by the Nanotherics MagneTherm system and the

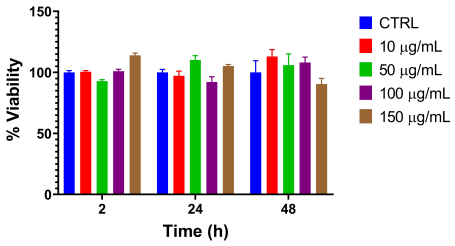

Figure 1: Biocompatibility of G-M55. MTT assay in MDA-MB-231 cells shows that G-M55 is safe up to a concentration of 150 µg/mL and up to 48 h of incubation time; the error bar represents SEM over six replicates.

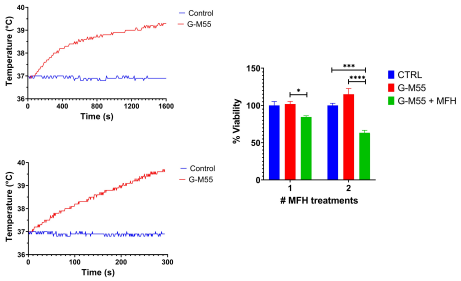

Figure 2: Efficacy as MFH agent. Thermograms acquired in water solutions of G-M55 using the Nanotherics Magneterm system (a) and the HYPER Module in MPI (b). c)MTT assay demonstrates statistically significant decrease of cell viability after one (*) and two (**) MFH treatments (24 h apart).

HYPER module in MPI, respectively.

The efficiency of G-M55 as MFH mediator was tested in MDA-MB-231 cells. Viability of MDA-MB-231 cells was strongly reduced when, after 24h of incubation with G-M55 (150 mg/mL), cells were subjected to two MFH treatments (see Figure 2). After two MFH treatments, cells viability was strongly reduced compared to both CTRL and cells incubated with G-M55 nanoparticles but not subjected to MFH treatments. Immunohistochemistry confirmed overexpression of heat shock protein (HSP70) in MDA-MB-231 cells treated with M-G55 and MFH.

To assess the ability of G-M55 to serve as a MPI contrast agent, images of MDA-MB-231 cancer cells were acquired (see Figure 3). At first, the signal intensity of G-M55 was acquired as a function of iron concentration in water solution. Figure 3a shows that the signal intensity linearly increases with iron concentration. Then, the signal intensity of MDA-MB-231 labeled with G-M55 was acquired as a function of the number of cells. Figure 3b shows that the signal intensity increases with the number of cells and that the detection limit is 30kcells (Figure 3c).

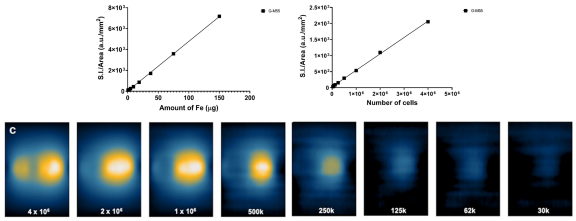

Figure 3: Efficiency of G-M55 as cellular imaging contrast agent in MPI. The straight line defines the ability of the tracer to serve as an efficient cell labeling agent in MPI. a) MPI signal intensity of G-M55 in water as a function of the amount of Fe per 100 µl of solution. b) MPI signal intensity as a function of the number of cells. c) MPI images of labelled cells.

IV. Conclusions

This study characterized an innovative nanomaterial, M55, as a contrast agent for MRI and MPI and as a therapeutic agent in MFH. A double shell, organic coating of citrate and glucose made the ferrite core water soluble and biocompatible. It was tested as a MFH agent in a triple negative human breast cancer (MDA-MB-231) cell line. Despite relative low SAR values in water solution, this agent was highly efficient in decreasing cell viability after two MFH treatments. Interestingly, similar SAR values were determined by using a standard equipment and a MPI apparatus, paving the way to its application in localized thermotherapy [3]. Further developments will be devoted to demonstrate the efficacy of G-M55 as a therapeutic agent in experimental model of breast cancer in vivo.

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Author's statement

Conflict of interest: JG, JM and PG are employed in Magnetic Insight Inc., Other authors state no conflict of interest. Informed consent: Informed consent has been obtained from all individuals included in this study.

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