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Research Article

Elaboration of Superparamagnetic and Bioactive Multicore–Shell Nanoparticles (γ-Fe_2O_3@SiO_2-CaO): A Promising Material for Bone Cancer Treatment

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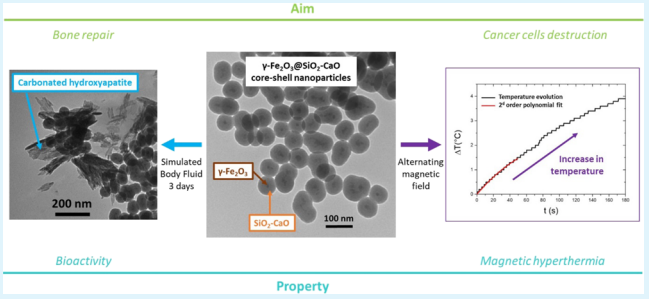
ABSTRACT: The past few decades have seen the development of new bone cancer therapies, triggered by the discovery of new biomaterials. When the tumoral area is small and accessible, the common clinical treatment implies the tumor mass removal followed by bone reconstruction or consolidation with a bioceramic or a metallic scaffold. Even though the treatment also involves chemotherapy or radiotherapy, resurgence of cancer cells remains possible. We have thus designed a new kind of heterostructured nanobiomaterial, composed of SiO_2-CaO bioactive glass as the shell and superparamagnetic γ-Fe_2O_3 iron oxide as the core in order to combine the benefits of bone repair thanks to the glass bioactivity and cancer cell destruction through magnetic hyperthermia. These multifunctional core–shell nanoparticles (NPs) have been obtained using a two-stage procedure, involving the coprecipitation of 11 nm sized iron oxide NPs followed by their encapsulation inside a bioactive glass shell by sol–gel chemistry. The as-produced spherical multicore–shell NPs show a narrow size distribution of 73 ± 7 nm. Magnetothermal loss measurements by calorimetry under an alternating magnetic field and in vitro bioactivity assessment performed in simulated body fluid showed that these heterostructures exhibit a good heating capacity and a fast mineralization process (hydroxyapatite forming ability). In addition, their in vitro cytocompatibility, evaluated in the presence of human mesenchymal stem cells during 3 and 7 days, has been demonstrated. These first findings suggest that γ-Fe_2O_3@SiO_2-CaO heterostructures are a promising biomaterial to fill bone defects resulting from bone tumor resection, as they have the ability to both repair bone tissue and act as thermoseeds for cancer therapy.



KEYWORDS: *bioactive glass, superparamagnetic nanoparticles, sol–gel, coprecipitation, magnetic hyperthermia, bone regeneration, iron oxide*

INTRODUCTION

Nowadays, cancer is one of the major causes of death. It is estimated by the World Health Organization (WHO) that the number of people with this pathology will increase by 75% in the next two decades. If not detected sufficiently early, the primary tumor cells spread through the blood vessels and form new cancerous colonies (metastases) in different parts of the body. Among the organs affected, bones are the third-privileged site after the lungs and the liver.[1] As a consequence, a rapid resorption of the bone mass takes place because of an unbalanced bone remodeling process.[2] The patients can thus experience serious pain and pathological fractures which are highly detrimental for their quality of life.[3] Currently, chemotherapy, radiotherapy, and surgery with bone reconstruction or consolidation are the most common clinical treatments.[4] Despite great progress, these approaches are complicated, with numerous side effects[5] and the patient survival rate is quite low especially for young people dealing with malignant bone tumors.[6] The development of alternative therapies that are more effective and less harmful is thus required.

Thanks to the development of nanotechnology, the past decades have seen significant breakthroughs in cancer therapies. Nanoparticle (NP)-mediated magnetic hyperthermia (MH) is one of the promising techniques for deep-seated tumor (such as bone tumor) treatment, as it exploits the tumor cell sensitivity to an increase in temperature over the physiological range. Under an alternating magnetic field (AMF), the magnetic NPs release heat in the tumor region

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which endamage the cancer cells.[7] Typically, a temperature between 41 and 46 °C alters the biological functions and physiological environment of the malignant cells leading to cellular death by apoptosis,[8] while a temperature above 46 °C induces their necrosis.[9,10] Iron oxide NPs (magnetite, Fe_3O_4, and maghemite, γ-Fe_2O_3) are the most promising candidates as thermoseeds for MH since the first experiments made on dog lymph nodes in 1957 by Gilchrist and co-workers.[11] They exhibit outstanding magnetic properties which allow them to have a high heating efficiency while being chemically stable in physiological media.[12] Furthermore, it is now well established that they are not toxic from a variety of studies based on their use in diagnosis and drug delivery.[13] Below about 30 nm in diameter, noninteracting iron oxide NPs are in a superparamagnetic state at ambient temperature, a magnetic behavior which is characteristic of monodomain ferro(i)-magnetic NPs above their blocking temperature (T_B).[9] In this state, heat generation under an AMF is the consequence of two relaxation mechanisms: (i) Néel relaxation, where magnetization reversal along the easy axis induces a power loss and (ii) Brown relaxation in which the NPs physically rotate in order to align their magnetization along the magnetic field direction, with heat release through friction.[9] Another specificity of superparamagnetic iron oxide NPs (SPIONs) is that no magnetization is measured in the absence of a magnetic field (remanence and coercivity are zero), which is crucial for in vivo applications as residual magnetization induces NP aggregation, leading to a net reduction of their heating efficiency.[14] Note that the individual superparamagnetic NPs do have a permanent magnetic moment, but in the absence of a magnetic field, its fluctuation along the easy axis induces a zero net magnetization. For all these reasons (heating efficiency, nontoxicity, and biodegradability), SPIONs are nowadays preferential thermoseeds for MH as evidenced by the first adjuvant/therapeutic treatment commercialized in Europe by Magforce Nanotechnologies.[15]

As already pointed out, bone cancer treatments also include bone consolidation or repair. New therapy approaches should thus encompass both tumor destruction and bone regeneration. Bioactive glasses are attractive materials for bone regeneration as they chemically and strongly bind with the surrounding tissues when implanted in a bone defect and offer a biocompatible surface for bone tissue regrowth.[16] This biological linkage is achieved through the formation of a hydroxyapatite (HAp) layer onto their surface, the composition of which is close to the one of the mineral bone matrix.[16] Traditionally composed of SiO_2, Na_2O, CaO, and P_2O_5 and elaborated by fusion (between 1000 and 1400 °C), bioactive glasses have been clinically used as an ossicle replacement in the eighties and more recently as bone fillers for small bone defects.[17] Lately, it has been reported that for a same composition, glasses in the ternary (SiO_2-CaO-P_2O_5) and binary (SiO_2-CaO) systems synthesized by the sol−gel process exhibit a higher bioactivity (HAp forming ability) than the one produced through the traditional high-temperature fusion route.[18] According to these authors, this result is related to their remarkable textural properties (high specific surface area arising from their porosity, about 150 m^2/g, and high surface/volume ratio) which are intrinsic of the sol−gel polymerization process. Several experimental findings suggest that the specific surface area is the main parameter controlling the glass bioactivity. For example, Fan et al.[19] showed that the biomineralization process is enhanced for spherical 58S bioactive glass NPs (BGNs) (60%mol SiO_2, 36%mol CaO, and 4%mol P_2O_5) because of their large contact surface. Lei et al.[20] also reported that sol−gel-based BGNs exhibit a higher bioactivity with respect to microsized ones. Beside the advantages of the nanometric size, several in vivo studies in the literature highlighted the benefits of bioactive glass for bone repair in comparison to other bone graft substitutes such as HAp and/or apatite−wollastonite glass ceramic.[21−23] Indeed, a superior percentage of bone ingrowth along with a higher quality was observed, which might be attributed to the effect of the dissolution products (soluble silica and calcium ions) that stimulate the osteogenic cells to trigger the bone matrix mineralization. For all the above mentioned reasons, the elaboration of BGNs is of great interest for osseous reconstruction.

New therapeutic approaches, based on the use of the remarkable characteristics of iron oxide and BGNs, are thus interesting to investigate for bone cancer treatment as they would combine both the benefits of bone repair and cancer cell destruction through MH. Up to now, studies on bioactive and magnetic materials have been mostly focused on the synthesis of magnetic glass monoliths through several methods which do not allow for the fine control of their properties.

For example, Bretcanu et al.[24] reported the synthesis of ferrimagnetic glass ceramic materials in the system SiO_2-Na_2O-CaO-P_2O_5-FeO-Fe_2O_3 by a melting procedure. They showed that the temperature used for melting controls the magnetic properties of the samples. However, their findings are not encouraging as the best sample elaborated exhibits a low heating capacity because of the crystallization of nonmagnetic hematite within the silicate matrix. In the work of Wang et al.,[25] the Fe_2O_3-CaO-SiO_2 glass ceramic elaborated by the sol−gel method and sintered at 950 °C for 1 h exhibits a rather low bioactivity if nucleating agents such as P_2O_5 and TiO_2 are not added. Abbasi et al.[26] elaborated different bioactive ferrimagnetic glass ceramic materials by doping a solid state-derived 45S5 bioglass matrix with a variable proportion of sol−gel based strontium hexaferrite particles ($SrFe_{12}O_{19}$, from 5 to 20%wt). However, this study was not able to demonstrate efficiency for bone cancer therapy as the composite materials exhibit poor magnetic and bioactive properties.

In this work, magnetic and bioactive core−shell NPs with a core composed of superparamagnetic maghemite NPs and a bioactive glass (SiO_2-CaO) shell have been synthesized by a combination of coprecipitation process and sol−gel chemistry. The main characteristics of these heterostructures in terms of physical features (size distribution, morphology, and specific surface area), along with their magnetic and bioactive properties have been evaluated and their heating capacity has been assessed under an external AMF. In addition, their cytocompatibility has been investigated in the presence of human mesenchymal stem cells (h-MSCs). To the best of our knowledge, this is the first time that such a multifunctional material, with few components, controlled physicochemical features and very interesting bioactive and magnetic properties have been obtained.

MATERIALS AND METHODS

Materials. Tetraethyl orthosilicate (TEOS, 99%), ammonium hydroxide solution (NH_4OH 28.0−30%), calcium nitrate tetrahydrate ($Ca(NO_3)_2{\cdot}4H_2O$), iron (II) choride tetrahydrate ($FeCl_2{\cdot}4H_2O$, 99%), iron (III) chloride hexahydrate ($FeCl_3{\cdot}6H_2O$, 99%), nitric acid solution (HNO_3 65%), and hydrochloric acid solution (HCl 32%)

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were purchased from Sigma-Aldrich. Absolute ethanol (EtOH 99.5%) and acetone were obtained from VWR Chemicals. Citric acid monohydrate (99.5%) was purchased from Labogros.

Synthesis of Maghemite (γ-Fe_2O_3) NPs. A colloidal suspension of maghemite NPs was prepared using the protocol already described by Vichery et al.[27] First, 11.4 mL of concentrated NH_4OH (14.8 M) was quickly poured into 36 mL of Fe(II) and Fe(III) chlorides acidic solution under vigorous stirring (pH = 0.06, [Fe(II)] = [Fe(III)] = 0.54 M). The iron precursors coprecipitate instantly to form magnetite (Fe_3O_4) particles which were recovered by magnetic decantation, washed twice with deionized water, and then dispersed in 4.9 mL of HNO_3 (2 M); 12 mL of a $Fe(NO_3)_3$ aqueous solution (1.5 M) were then poured onto the flocculate and the mixture was heated up to reflux for 30 min in order to fully oxidize magnetite into maghemite. Finally, the iron oxide particles were recovered and washed three times with acetone prior to their peptization in 30 mL of an aqueous nitric acidic solution (pH = 2). A sonication (10 min) step and two centrifugation steps (6490 g/5 min) were performed in order to reduce the particle size dispersion.

Maghemite NP Functionalization. The smaller the particle size, the higher their surface reactivity.[13] Hence, the surface of the coprecipitated iron oxide particles was functionalized with citric acid to prevent their agglomeration. Citric acid is well known to enhance the colloidal stability of many oxide-based NPs by electrostatic repulsion in a large range of pH.[28] To do so, 15 mL of an aqueous citric acid solution (0.3 M) were added to 0.97 mL of the previously obtained ferrofluid (concentration of 73 mg_{Fe2O3}/mL) and the resulting solution (pH = 4.2) was kept under stirring during 30 min. After three magnetic decantation/washing steps with acetone, the functionalized particles were dispersed by peptization in H_2O/NH_4OH (5 mL/0.5 mL). The resulting solution is called solution 1.

Synthesis of Heterostructured NPs (γ-Fe_2O_3@SiO_2-CaO). The growth of the bioactive glass shell around the maghemite NPs was performed following a modified Stöber route. First, two solutions were prepared separately at room temperature: solution 2 corresponds to 6.1 mL of TEOS (0.14 M) + 100 mL of EtOH, and solution 3 to 2 mL of concentrated NH_4OH (14.8 M) + 58.5 mL of H_2O + 87.5 mL of EtOH. After 10 min stirring, solution 1 (citrated maghemite colloid) was quickly poured into solution 3 under constant stirring. After 10 min, solution 2 was added to the previous mixture and the resulting solution was kept under stirring for 3 h. Then, 1.9 g of $Ca(NO_3)_2.4H_2O$ dissolved in 2 mL of deionized water were added and the final solution was stirred for 21 h. A brown precipitate was collected by centrifugation (6297 g/ 10 min) and washed several times with deionized water until a colorless supernatant was obtained. Finally, the recovered sample was dried in an oven at 60 °C and annealed in air at 650 °C for 3 h in order to promote the diffusion of Ca^{2+} ions inside the silica network.

Physicochemical Characterization. The morphology of bare maghemite and heterostructured NPs was characterized using a Hitachi H-7650 transmission electron microscope operating at 80 kV, on powder samples first dispersed in deionized water and deposited onto a transmission electron microscopy (TEM) grid. More than 200 particles were systematically analyzed using ImageJ software to determine particles size distributions.

High-resolution transmission electron microscopy (HRTEM) experiments were performed using a Titan Themis G3 field emission gun electron microscope operating at 300 kV with a C-Twin polar piece (Cs = 2.7 mm, Cc = 2.7 mm, point resolution = 0.14 nm). Images were acquired on a 4096 × 4096 pixels CETA II CMOS camera with an electron dose of d ≈ 150 electrons.$Å^{-2}.s^{-1}$ at 660,000X magnification.

X-ray diffraction (XRD) patterns were recorded in the range 2θ = 20−80° with a step of 0.016° using a PANalytical X'Pert Pro diffractometer for bare iron oxide particles and a D2 phaser (Bruker) diffractometer for the heterostructured samples, with the two instruments mounted in the Bragg−Brentano configuration and equipped with a Cu anode ($\lambda_{K\alpha1}$ = 1.5406 Å, $\lambda_{K\alpha2}$ = 1.5444 Å). The instrumental resolution function was obtained from a LaB_6 NIST standard (SRM 660a).

The internal structure of the samples was also characterized by Fourier transform infrared spectrometry (FTIR, Nicolet 5700, Thermo Scientific) in transmission mode between 1400 and 400 cm^{-1}. Measurements were performed on pellets made of KBr and particles in a weight ratio of 199:1.

The adsorption−desorption isotherms were recorded with a Micromeritics Tristar II PLUS sorptometer. The Brunauer−Emmett−Teller equation was applied to calculate the specific surface area.

The sample composition was determined by inductively coupled plasma-atomic emission spectroscopy (ICP-AES) using a ULTIMA-C spectrometer. A mixture of the sample powder (100 mg) and $LiBO_2$ (300 mg) was melted for 5 min at 1100 °C in an induction furnace. The obtained melt droplets were then dissolved in HNO_3 (50 mL, 1 M) and the volume of the solution was completed to 200 mL with 1 M HNO_3. Reference materials were prepared in the same way, while a pure $LiBO_2$ solution (300 mg in 200 mL 1 M HNO_3) was used as blank. The analytical wavelengths used for Si and Ca are λ = 251.611 nm and λ = 317.933 nm, respectively.

The zeta potential was measured using a Zetasizer nano apparatus (Nano-ZS, Malvern Instruments), after redispersion of the powder (citrated iron oxide) in deionized water and sonication for 10 min.

Magnetization measurements were performed on powder samples using a SQUID magnetometer (Cryogenic SX600). The samples were wrapped in food-grade transparent plastic film, which diamagnetic contribution was systematically subtracted. The fraction of magnetic material in each sample was derived from the iron content measured by ICP-AES. Magnetization versus magnetic field curves were recorded at 300 and 10 K and zero field cooled-field cooled (ZFC-FC) measurements were performed under 25G. The ZFC branch was obtained by cooling the sample down to the lowest temperature achievable by the magnetometer under a zero magnetic field. Then, a small external magnetic field (25 G) was applied and the magnetization of the sample was recorded upon heating up to room temperature. The FC branch has been obtained in the same way, except that the cooling step was performed under 25 G.

In Vitro Bioactivity Study. The apatite-forming ability was evaluated using the method reported by Kokubo et al.[29] Typically, 50 mg of sample powder were dispersed in a plastic beaker containing 50 mL of simulated body fluid (SBF), an alkaline solution mimicking the inorganic composition of human blood plasma, and kept in an orbital shaking incubator at 37 °C (N-BIOTEK). After 3 days of soaking, the particles were recovered by centrifugation, gently washed twice with deionized water, and finally dried at 60 °C before further characterization.

Measurements of Specific Absorption Rate− MH Experiments. Specific absorption rate (SAR) measurements were performed by a calorimetric method conducted on a MH apparatus (DM 100 instrument and DM applicator, Nanoscale Biomagnetics TM, associated with MaNIaC software). An adapted glass vial filled with 1 mL of an aqueous dispersion containing maghemite (powder) or heterostructured (powder/pellet) NPs with a Fe concentration of 1 mg/mL was subjected to AMF (H = 300 G and f = 536.5 kHz) and the thermal profile was monitored over 5 min.

Cell Culture. h-MSCs were extracted from metaphysic cancellous bones collected during hip arthroplasty surgical procedures on patients who had previously signed an authorization for the use of their bones for research purposes. The bones were collected in a solution of sterile phosphate-buffered saline (PBS) supplemented with 2% of heparin and transported immediately to the cell culture laboratory. After being washed with PBS, they were cut into small pieces and incubated for 15 min at 37 °C with 6 mL of minimum essential media (MEM) and 0.2 mL of collagenase. Then, bone pieces were filtered and washed with PBS in order to recover the h-MSCs, which were subsequently suspended in a standard marrow cell culture medium composed of MEM supplemented with gentamycin, sodium pyruvate, vitamins, nonessential amino acids, and fetal bovine serum. Cells were plated at 20 × 10^6 cells in 25 cm^2 tissue culture flasks and incubated at 37 °C with 5% of humidified CO_2. After 3 days, the flasks were gently rinsed twice with PBS to remove the nonadherent cells.

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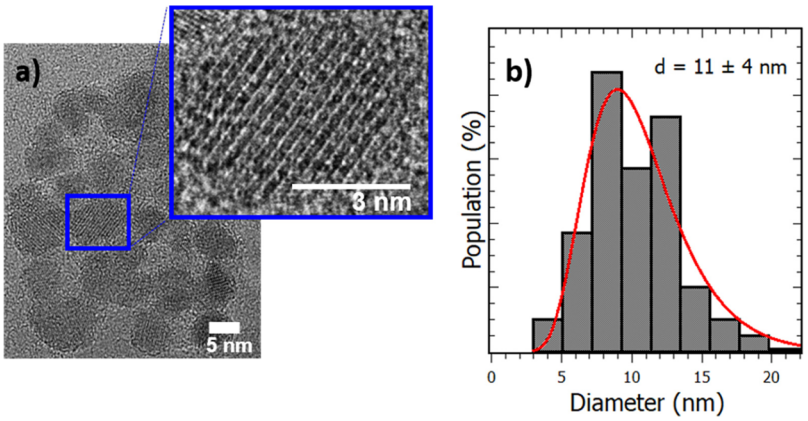

Figure 1. (a) Representative HRTEM image with a magnification in the inset and (b) size histogram of the magnetic NPs with the mean value and the variance of the distribution.

Adherent h-MSCs were fed by a weekly change of medium and expanded through one of three passages before being collected by trypsinization.

In Vitro Cytotoxicity Tests. The in vitro cytotoxicity tests were performed using the MEM cultured h-MSCs and sterilized samples (kept in an oven at 180 °C for 2 h). h-MSCs were seeded in a 24-well plate (5×10^4 cells/well), exposed to sample powders (concentration of 1 mg/mL) and then incubated for 1 week at 37 °C. h-MSCs cultured without powder were used as a control. After 7 days, the mitochondrial activity of the cells was evaluated using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assays. For this test, 100 μL of the MTT reagent at a concentration of 5 mg/mL in PBS was added to each well and the culture plates were incubated at 37 °C with 5% of CO_2. After 3 h, the content of each well was carefully removed, leaving the powder and the cells at the bottom of the wells. Dimethyl sulfoxide (500 μL) was then added in each well to lyse the cells and release the staining induced by the MTT reagent. After complete dissolution, the optical density (OD) of the wells was measured at 570 and 690 nm (spectrophotometer TECAN).

Statistical Analysis. Statistical analysis was performed using the Mann−Whitney nonparametric test with the Bonferroni correction, $p < 0.05$ being considered as statistically significant. All of the experiments were performed in triplicate.

RESULTS AND DISCUSSION

Elaboration, Morphology, and Structural Characterization. This study proposes the growth of a bioactive shell around preformed magnetic NPs to obtain bioactive and magnetic heterostructures. One major advantage is the possibility to finely control and study separately the magnetic properties of the bare magnetic particles.

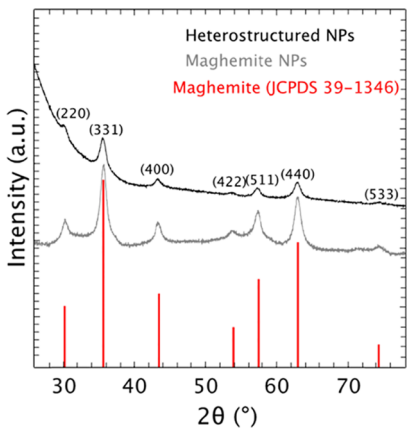

Figure 2. Overlay of the maghemite (gray, bottom) and the heterostructure (black, top) diffraction patterns.

Maghemite NPs were elaborated using a two-step procedure involving precipitation from Fe(II) and Fe(III) salts under alkaline conditions and then the oxidation of the magnetic colloid under reflux in the presence of $Fe(NO_3)_3$ used as a mild oxidizing agent. HRTEM images show that the particles exhibit a spheroidal morphology characteristic of the synthesis protocol (see Figure 1).[13] The average diameter evaluated by fitting the size histogram with a log-normal function is 11 nm (size dispersion of 36%). Thus, magnetic NPs with only a fair polydispersity in size have been obtained, presumably because of an overlap of the nucleation and growth processes during the addition of ammonia as magnetite formation follows the prediction of classical nucleation theory.[30−32] The observation of lattice fringes through the whole grain (see inset of Figure 1a) suggests that the particles are monocrystalline and present few defects.[33]

The diffraction pattern of the bare iron oxide particles shows Bragg peaks characteristic of a spinel structure, either maghemite (γ-Fe_2O_3) or magnetite (Fe_3O_4), see Figure 2. As the lattice parameter a differs for the two ferrites, its value has been accurately determined by fitting the experimental profile by full pattern matching (see Figure S1 in Supporting Information). The refinement yields $a = 8.354 \pm 0.005$ Å, which is close to the theoretical lattice constant of natural maghemite ($a = 8.352$ Å, JCPDS 39−1346) and quite far to the one of magnetite ($a = 8.396$ Å, JCPDS 19−0629). Note that for a similar synthesis, Mössbauer spectroscopy experiments have confirmed the complete oxidation of the iron oxide particles.[27] Thus, it can be concluded that maghemite NPs have been obtained. Moreover, the coherence length evaluated from the (331) Bragg peak using the Scherrer formula is of about 9 nm, in good accordance with the HRTEM observations, confirming the monocrystalline character of the particles.

The formation of a bioactive shell around the maghemite NPs has been achieved through a modified Stöber method, which first required a functionalization of the particles with citric acid to ensure their dispersion in alkaline media prior to the sol−gel process. Once the silica shell is formed, the calcium salt is added into the reaction media. The bioactive SiO_2-CaO shell is obtained after washing steps to remove counterions and unreacted species, and a thermal treatment at 650 °C to

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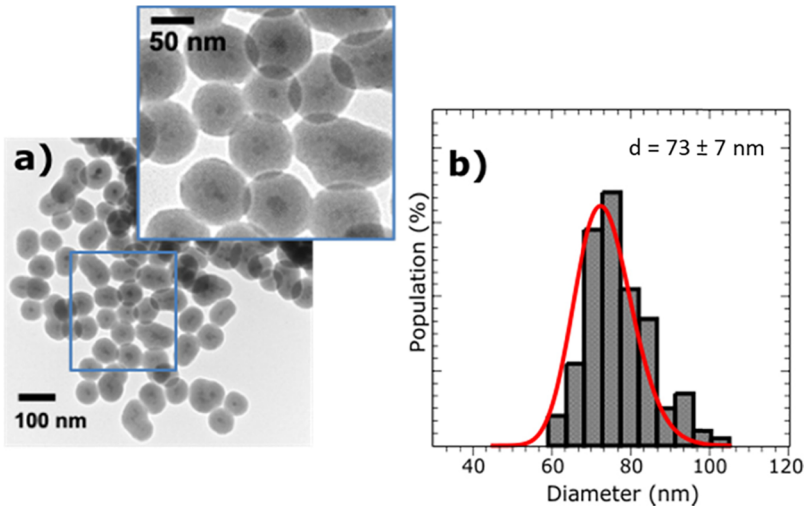

Figure 3. (a) Low-magnification TEM image and (b) size histogram of the γ-Fe_2O_3@SiO_2-CaO heterostructures.

Table 1. Nominal and Actual Composition of the Heterostructured Sample

	SiO_2	CaO	Fe_2O_3
nominal composition (%molar)	76.2	22.4	1.4
actual composition (%molar)	86.2 ± 0.7	12.8 ± 0.1	1.02 ± 0.01

promote calcium diffusion and obtain a more uniform calcium distribution within the silica glass. This two-step process with a separate Ca incorporation allows avoiding side effects on the final particle textural properties (aggregation, nonsphericity, and high polydispersity in size) as reported in many studies.[34−36] Representative TEM micrographs of the heterostructured sample show that the composite particles exhibit a spherical shape with a multicore−shell structure (see Figure 3a). Such a result could be explained by the relatively good colloidal stability of the maghemite NPs in the reaction bath. Indeed, the functionalization of the magnetic NPs by citrate molecules induces an increase of the net surface charge because of the deprotonation of up to three carboxylic groups (pKa values of 3.13, 4.76, and 6.40 for citric acid). Thus, a rather good electrostatic repulsion between the citrated magnetic NPs can be obtained as evidenced by their zeta potential value of −29 mV. Hence, one can see in Figure 3a that the bioactive glass shell has grown mostly around small

Table 2. Weight Percentage of Maghemite, Magnetization Saturation (M_s) at 300 K, and Coercive Field (μ_0H_c) at 10 K for the Heterostructures and the Bare Maghemite Particles

sample	%wt Fe_2O_3	M_s (300 K) (emu/g_{Fe2O3})	μ_0H_c (10 K) (G)
γ-Fe_2O_3	100	55 ± 2	210
γ-Fe_2O_3@SiO_2-CaO	3.65 ± 0.02	57 ± 1	210

aggregates and for a part around single particles. From the TEM images, we can also infer that no homogeneous nucleation of silica particles occurs and that all the magnetic particles are encapsulated. As illustrated in Figure 3b, the heterostructures exhibit a mean size of 73 and a size dispersion about 10%, proving that they are nanosized and quite monodispersed. In addition, they are nonagglomerated which means that the thermal treatment and the calcium salt addition in the reaction media 3 h after the beginning of the sol−gel process had no significant impact on their mean size and agglomeration state, as reported in our previous studies.[37,38] N_2 adsorption experiments (see Figure S2 in Supporting Information) showed that the heterostructures are not porous and display a specific surface area of 36 m^2/g, which is very close to the theoretical value (S_{theo} = 37 m^2/g) calculated using

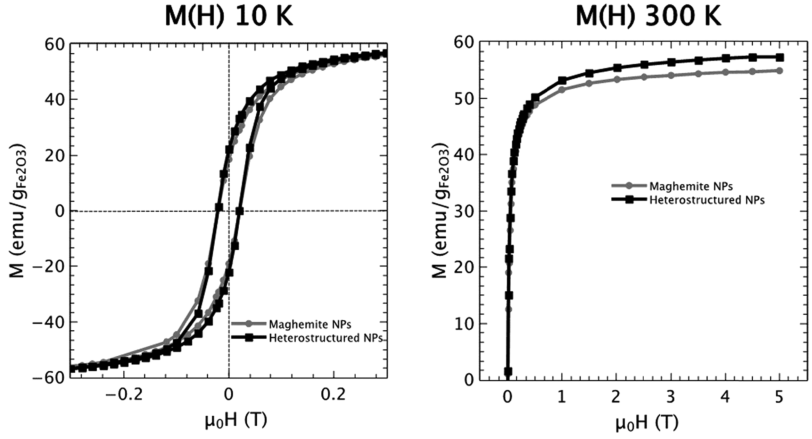

Figure 4. M(H) curves of maghemite (gray, circles) and heterostructured (black, square) NPs at 10 and 300 K.

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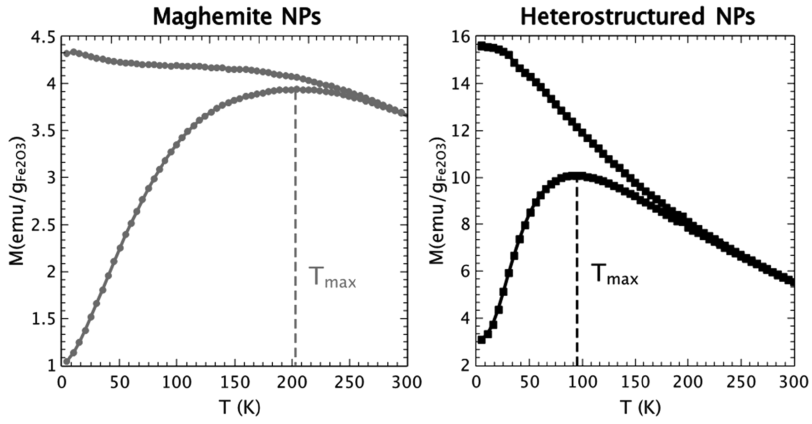

Figure 5. ZFC-FC magnetization curves of maghemite and heterostructured NPs recorded at 25 G.

Table 3. SAR Values, ILP Values, and Temperature Elevation after 1 min under AMF (ΔT) for Maghemite NPs (Colloidal Dispersion) and Heterostructured NPs (Colloidal Dispersion and Pellet Sample)

sample	SAR (W/g_{Fe})	ILP ($nH.m^2/kg$)	ΔT (°C)
γ-Fe_2O_3 (colloidal dispersion)	757 ± 5	2.49 ± 0.01	7.5
γ-Fe_2O_3@SiO_2-CaO (colloidal dispersion)	159 ± 5	0.52 ± 0.01	1.5
γ-Fe_2O_3@SiO_2-CaO (pellet)	122 ± 5	0.40 ± 0.01	1.3

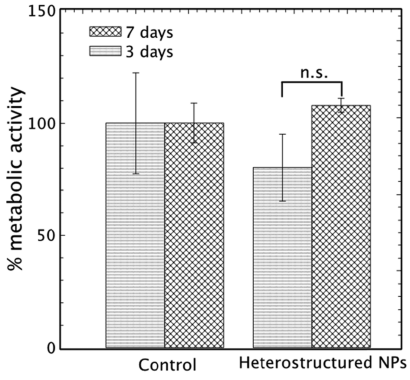

Figure 6. MTT results after incubation of the heterostructured NPs with h-MSCs during 3 and 7 days, (* p > 0.05). n.s.: not significant.

their size histogram. This hence confirms their nonagglomeration state.

As displayed in Figure 2, the XRD pattern of the heterostructured particles exhibits both a strong background corresponding to the diffuse scattering of the glass network and the characteristic peaks of maghemite. No other crystalline phase (like calcium and/or iron silicates) is evidenced confirming the chemical purity of the sample.

Similar to what was observed for the bare maghemite NPs, the Scherrer equation applied to the (331) diffraction peak leads to a coherence length value of about 10 nm, indicating the absence of significant particle coarsening on heating at 650 °C. It is important to point out that a sintering of bare maghemite particles occurs for annealing temperatures above 400 °C, leading to their phase transformation into hematite.[27] As no hematite peak can be detected in the heterostructures diffractogram, we can conclude that the iron oxide multiple cores within a SiO_2-CaO particle should be separated from each other by a thin silica layer that prevents their coarsening and thus their phase transition into hematite.

Table 1 summarizes the heterostructures composition obtained by ICP-AES. About 57% of the initial Ca^{2+} ions have been incorporated in the glass network, an insertion rate consistent with our previous findings.[37]

Magnetic Properties. Magnetization versus magnetic field curves were recorded at 10 and 300 K for both the maghemite and heterostructured NPs. The magnetization values were normalized with respect to the iron oxide mass in each sample. Figure 4 shows similar magnetic behavior for the single-phase and composite samples. Their M(H) curves at 10 K exhibit a small coercivity (see Table 2) while those recorded at 300 K present no hysteresis loop. The latter magnetic behavior is

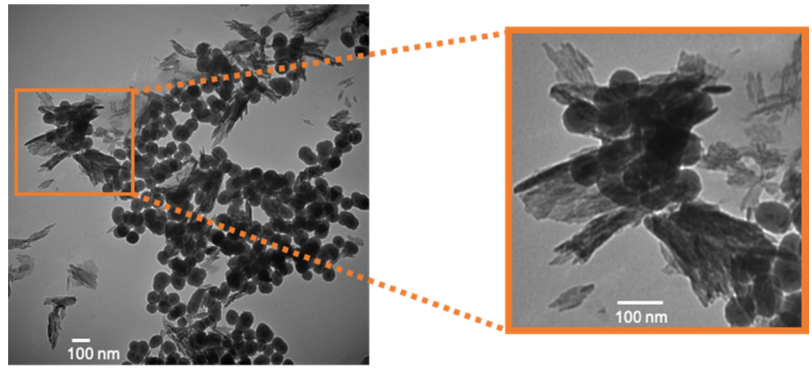

Figure 7. TEM image of the heterostructured NPs after 3 days of immersion in SBF.

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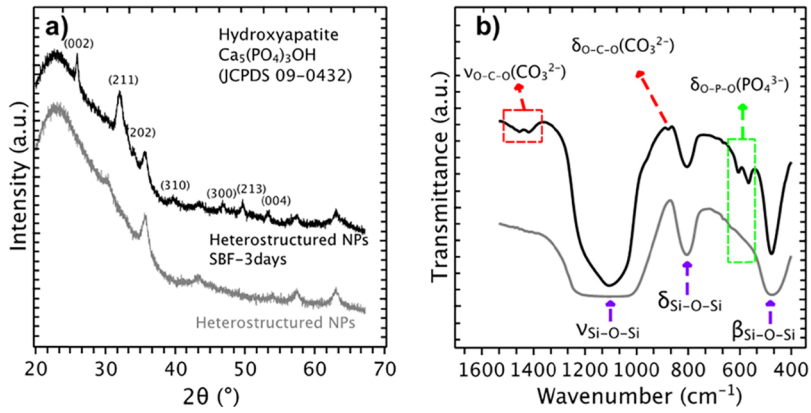

Figure 8. (a) XRD profiles and (b) FTIR spectra of heterostructured NPs before (gray, bottom) and after (black, up) 3 days of immersion in SBF.

characteristic of particles in a superparamagnetic state.[39] The saturation magnetization (M_S) values at 300 K (see Table 2) are much lower than the one of bulk maghemite (M_S = 75 emu/g) because of an enhanced contribution of disordered surface spins (spin canting) as already described in other studies.[33] Also, one should note the M_S values for the bare magnetic particles and the heterostructures are not significantly different considering the uncertainties of weight measurements and determination of the Fe_2O_3/SiO_2-CaO mass ratio, meaning that the magnetic properties of the iron oxide particles have not been significantly altered by the growth of the shell and by the post-synthesis heat treatment. The M_S of the heterostructured NPs is of 2.09 ± 0.04 emu per gram of sample (see Figure S3 in Supporting Information). It is interesting to compare this M_S value to those of other multifunctional materials described in the literature. A sol−gel derived bioactive glass mesoporous monolith with 3%mol of iron presents a *M*s value of 0.15 emu/g,[40] another one with 5% mol of iron has a M_s value of 0.21 emu/g,[41] a mesoporous bioactive glass scaffold with 5%mol of iron exhibits a M_s value of 0.2 emu/g,[42] and another study presents one with a maximum value of 1.75 emu/g for a 9%mol of iron loading.[43] For all these composite materials, the iron precursor has been introduced during the sol−gel process and formation of the magnetic NPs occurred during a thermal treatment. There was thus no control of their nucleation and growth, leading to very small or poorly crystallized particles, and hence lower M_S values, despite a larger amount of iron compared to the heterostructures presented here (2.8%mol of Fe). Liu et al. synthesized a multifunctional magnetic mesoporous bioactive glass with 5%wt of magnetic NPs in a two-step process, first synthesizing the magnetic particles and then incorporating them in an acidic bioactive glass sol.[44] Despite the large M_s value of the bare magnetic NPs (59 emu/g), the composite sample only presents a M_S value of 1.44 emu/g. The authors tentatively assigned this finding to a partial dissolution of the magnetic NPs during the acidic hydrolysis step of the sol−gel process. In the present study, as the bioactive glass shell was grown in alkaline media, the integrity of the particles was preserved so that they retained their original magnetic properties. Note that the M_S value of the heterostructured NPs can be further increased to some extent by decreasing the thickness of the bioactive glass shell.

ZFC-FC measurements have also been performed to emphasize the transition from the blocked to superparamagnetic state when increasing the temperature. This transition is characterized by T_B, over which the thermal energy overcomes the barrier energy between two magnetization states, allowing the fluctuation of the coupled magnetic moments along the easy magnetic axis. This situation results in a zero average magnetization. Actually, the maximum of the ZFC curves (T_{max}) corresponds to the T_B only in the case of an assembly of noninteracting and monodisperse particles. T_{max} departs from T_B when the particles are polydispersed in size and when there are strong magnetic dipolar interactions between them.[45,46]

Figure 5 shows that the T_{max} value is quite different for the bare maghemite NPs and the heterostructures, with T_{max} values of 205 and 93 K, respectively. This discrepancy results mainly from different dipolar magnetic interactions which are also evidenced from the FC profiles. Indeed, the shape of the FC curves at low temperature is a good indication on the strength of these interactions as a plateau is characteristic of strongly interacting particles whereas a steep slope is specific of isolated particles.[47] FC curves thus confirm that the magnetic dipole interactions are far stronger for bare magnetic NPs than for the particles embedded in the SiO_2-CaO shell. Along with the noncoarsening of the magnetic particles after thermal annealing, this result gives another hint on the fact that the multiple iron oxide cores within a composite particle are separated by a thin silica layer.

SAR Measurements. The target application of these magnetic and bioactive heterostructures is to destroy cancer cells through MH. Calorimetric measurements under an applied AMF have thus been performed on aqueous dispersion of these magnetic heterostructures in order to assess the power dissipated by the particles, also called SAR or specific loss power. Measurements were carried out on bare maghemite particles and on heterostructures in order to investigate the impact of the SiO_2-CaO shell on heat release. The operating system not being under perfect adiabatic conditions (with thermal losses during measurement), the temperature profiles were recorded as a function of time and treated by means of the initial slope method (see Figure S4 in Supporting Information) in order to calculate the SAR. SAR is given per unit mass of iron ($W.g^{-1}$) and is expressed according to the relation:

$$\mathrm{SAR} = \frac{C_p}{m(\mathrm{Fe})}\left(\frac{\Delta T}{\Delta t}\right)_{t=0}$$

where C_p is the specific heat capacity of the magnetic suspension (here it corresponds to the one of water given

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the sample dilution), m(Fe) the mass of iron inside the sample, and $\left(\frac{\Delta T}{\Delta t}\right)_{t=0}$ the initial temperature rising rate.

The interdependence of SAR with the magnetic field parameters (magnetic field frequency and amplitude) makes it difficult to compare the heating efficiency of the samples reported in the literature. Therefore, an alternative parameter, the intrinsic loss power (ILP, proportional to the SAR via the relation below) is conventionally used for this purpose.[48]

$$\mathrm{ILP} = \frac{\mathrm{SAR}}{H^2 f}$$

where H and f are the magnetic field amplitude and frequency, respectively. However, this parameter is reliable only for relatively low magnetic field amplitudes and frequencies.

Table 3 presents the SAR and ILP values of the maghemite and heterostructured NPs subjected to an AMF (H = 300 G and f = 536.5 kHz) for 3 min. The rise in temperature after 1 min of applied AMF is also reported to help visualize the heating capacity.[49] The bare maghemite NPs exhibit SAR and ILP values about 5 times higher than the ones of the heterostructures. This can be explained for a part by the presence of magnetic dipolar interactions in the heterostructures. Indeed, when SAR measurements are performed on aqueous dispersions at low concentration (1 mg_{Fe}/mL), bare maghemite NPs are far away from each other and can be considered as noninteracting, whereas magnetic dipolar interactions should exist for the heterostructures as their core is composed of several Fe_2O_3 NPs at a close distance. The impact of magnetic dipolar interactions on the heating power can be positive or detrimental, depending on the spatial arrangement of the magnetic particles. In the case of facetted particles, under an external magnetic field, dipolar interactions may induce a chain-like arrangement which promotes an increase of effective anisotropy, thus enhancing the heating power of the material.[10,50,51] These interactions have also been shown beneficial in nanoflower structures in which the crystallographic orientation of each primary particle is the same.[52,53] For these two examples, the collective behavior of the particles was found to improve their thermal efficiency. In the case of the multicore−shell NPs studied here, magnetic dipolar interactions are expected to have a detrimental effect, because the individual maghemite particles present disordered crystalline orientations, as pointed out by previous studies.[54−56] In addition, the low thermal conductivity of the SiO_2-CaO shell induces a thermal shielding,[57] so that the heat generated by the magnetic NPs is not efficiently transferred to the surrounding media (silica heat capacity of 0.35 J/kg versus 4.18 J/kg for water). Nonetheless, interestingly, the ILP value of the heterostructures developed in this work lies in the range of already commercialized magnetic NPs (0.15−3.1 $nH{\cdot}m^2{\cdot}kg^{-1}$),[48] meaning they can be promising candidates for MH therapeutics.

Hyperthermia measurements were also performed on pellets made of pressed heterostructure powder in order to discriminate the contributions from Néel (heat produced by the magnetic relaxation of the particles' magnetization) and Brown (heat produced by the friction of the particles rotating in the media) relaxations. In the pellet configuration, particles are mechanically blocked so the only efficient mechanism is Néel relaxation.[58,59] Here, the SAR and ILP values summarized in Table 3 show a slight reduction for the pellet sample with respect to the colloidal dispersion. This result is consistent with the literature as it is generally agreed that below 15 nm, Néel relaxation is the predominant contribution to SAR.[60]

In Vitro Cytotoxicity. The second target to assess the potential of these multifunctional particles as new therapeutic agents is their ability for bone reconstruction. For such biomedical applications, composite samples require further biological investigations, especially a proof of their cytocompatibility. We thus performed in vitro cytotoxicity tests as it is admitted that a material which is not toxic in vitro will also not be toxic in vivo.[61] To do so, MTT assays were used to assess the viability of h-MSCs after incubation with the heterostructured NPs during 3 and 7 days, respectively. According to Figure 6, the metabolic activity of h-MSCs does not change significantly in the presence of the particles, an observation confirmed by statistical analysis ($p > 0.05$). Consequently, these heterostructured NPs can be considered as nontoxic.

In Vitro Bioactivity. The glass shell bioactivity was addressed by immersing the sample into SBF, a salt solution mimicking the inorganic part of human plasma and following the sample mineralization process (HAp formation).[62] Figure 7 shows a TEM image of the heterostructured NPs soaked in SBF for 3 days. A heterogeneous growth of crystals onto the particle surface can be observed. Their nature has been identified by XRD and FTIR spectroscopy. A closer inspection of the XRD pattern shown in Figure 8a shows new Bragg reflections characteristic of HAp (JCPDS 09−0432).[38] Furthermore, the presence of maghemite NPs peaks suggests their immobilization inside the heterostructures or within the apatite phase.

Although the FTIR spectrum of the raw heterostructured NPs only shows absorption bands of the silica network, the FTIR spectrum of the particles immersed in SBF clearly shows the appearance of two new bands, respectively, at 564 and 604 cm^{-1} (Figure 8b) which can be assigned to HAp.[63] In addition, one can notice the presence of three additional bands located at 879, 1419, and 1458 cm^{-1} which can be attributed to the O−C−O stretching and bending vibration of carbonate groups.[64,65] This indicates the formation of carbonated HAp, a mineral phase with a composition close to the one of natural bone. XRD and FTIR measurements thus prove that the heterostructured NPs elaborated in this study are bioactive. It should also be noted that in contrast to the works of Ebisawa et al.[66] and Ohura et al.,[67] the bioactivity of the nanocomposite is preserved despite the presence of iron oxide in the glass matrix.

CONCLUSIONS

In this study, superparamagnetic and bioactive NPs with a multicore−shell structure were designed through a multistep approach. Maghemite NPs were preformed by a simple coprecipitation route and functionalized with citric acid to enhance their colloidal stability prior to being coated with sol−gel silica (modified Stöber protocol). Finally, the addition of calcium ions in the solution and their diffusion within the silica network upon postsynthesis annealing allowed the formation of a bioactive glass shell. Physical characterization showed that the heterostructures are not agglomerated and retain a nanometric size with a low polydispersity (73 ± 7 nm). An investigation of their heating capacity under an external AMF pointed out that they induce a temperature rise of water efficient enough to have a therapeutic effect on cancer cells. Furthermore, the in vitro bioactivity tests confirmed that the multifunctional material is bioactive after 3 days of immersion

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in SBF, as evidenced by the precipitation of HAp on its surface. In vitro MTT assays in the presence of h-MSCs highlighted its cytocompatibility. Based on these findings, the elaborated γ-Fe2O3@SiO2-CaO heterostructures should be considered as promising for bone cancer treatment and should deserve further studies to improve their heating capacity by varying the size of the magnetic core and the synthesis parameter to obtain monocore−shell NPs with a thinner bioactive shell. Also, an experimental procedure which effectively demonstrates in vitro cancer cell destruction in the presence of such heterostructures in an AMF should now be conducted.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsami.0c12769.

Protocol for iron oxide lattice parameter determination; N_2 adsorption−desorption isotherm; M(H) curve of the heterostructured NPs plotted with the magnetization value in emu per gram of heterostructures and temperature evolution of bare maghemite NPs; heterostructured NPs and pure water under an AMF with H = 23.8 kA/m and f = 536.5 kHz along with fittings for SAR calculation (PDF)

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Author Contributions

X.K. carried out all the experiments except the cytotoxicity tests (Aurélie Jacobs), the magnetic measurements (I.M.), the HRTEM imaging (E.L.), and the SAR measurements (A.A. and D.M.) and processed all experimental data. C.V. designed the study and was in charge of overall direction and planning. All authors provided critical feedback and helped shape the research, analysis, and manuscript.

Notes

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■ ABBREVIATIONS

AMF, Alternating Magnetic Field
BGNs, Bioactive Glass Nanoparticles
BET, Brunauer−Emmett−Teller
DMSO, Dimethyl Sulfoxide
FTIR, Fourier Transform Infrared Spectroscopy
HAC, Hydroxyapatite Apatite Carbonated
HAp, Hydroxyapatite
h-MSCs, Human Mesenchymal Stem Cells
HRTEM, High-Resolution Transmission Electron Microscopy
ICP-AES, Inductively Coupled Plasma-Atomic Emission Spectroscopy
ILP, Intrinsic Loss Power
MEM, minimum essential media
MH, Magnetic Hyperthermia
M_s, Saturation Magnetization
MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide
NPs, Nanoparticles
OD, Optical Density
PBS, Phosphate Buffer Saline
SAR, Specific Absorption Rate
SBF, Simulated Body Fluid
SPIONs, Superparamagnetic Iron oxide Nanoparticles
T_B, Blocking Temperature
TEM, Transmission Electron Microscopy
TEOS, Tetraethyl Orthosilicate
WHO, World Health Organization
XRD, X-Ray Diffraction
ZFC-FC, Zero-Field Cooled-Field Cooled.

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