Magnetic Nanoparticles extracted from magnetotactic bacteria as contrast agents for MRI

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Target Audience

Biological and biomedical applications of chemically synthesized magnetic nanoparticles have often been hampered by biocompatibility problems. Naturally occurring iron-oxide nanoparticles, namely Magnetosomes (MS), are here proposed as theranostic agents for imaging and thermotherapy of tumors. The target audience for this work is represented by basic researchers working in the field of contrast agents for MRI, molecular imaging and tumor thermotherapy.

Purpose

Magnetotactic bacteria (MTB) form MS: specialized organelles that consist in membrane-enveloped and nano-sized crystals of magnetite (Fe3O4). Inside bacteria, individual MS are organized in chains that are used by bacteria as a compass for geomagnetic navigation. Interestingly enough, the first description of MTB appeared in 1963 in a publication of the Istituto di Microbiologia of the University of Pavia by Salvatore Bellini; a more complete report was later published in Science[1].It has been reported that MS extracted from MTB could act as theranostic agents: as magnetic fluids for r.f. magneto-thermotherapy and, at the same time, as contrast agent for MRI. We report here the thermal and contrastographic properties of MS extracted from *Magnetospirillum gryphiswaldence strain* MSR-1.

Methods

MS were extracted from M. *gryphiswaldence strain* MSR-1, purified according to methods published by Grunger[2] and therefore lyophilized for 5 h, irradiated with γ-rays 56Gy for 84 minutes and stored at -20° C. MR images were acquired using a Bruker tomograph operating at 4.7 T (Bruker, Germany). Relaxivities were measured in aqueous solution at different frequency (0.1 to 60 MHz) and compared to those of Endorem, a commercial contrast agent. The NMR signal detection and generation was obtained with a Smartracer® Fast-Field-Cycling relaxometer (Stelar, Mede, Italy) in the range 10 kHz–10 MHz and with a Stelar Spinmaster spectrometer in the range 10 MHz–60 MHz. Saturation Recovery and Carr Purcell Meiboom Gill (CPMG) pulse sequences were used for T1 and T2 measurements, respectively. Magnetic measurements were carried out on powders and suspensions using a Quantum Design SQUID MPMS XL-7 magnetometer. The zero field-cooled and field-cooled (ZFC/FC) curves were obtained with an applied magnetic field of 50 Oe in the temperature range 2–300 K, while the field dependent magnetization measurements were recorded in the range of ±5 T at both 2 K and room temperature. Whole mount bacteria or extracted MS were fixed with glutharaldehyde 2% in Sorensen buffer pH 7.4 for 2 h, post-fixed in 1% osmium tetroxide in aqueous solution for 2 h, dehydrated in graded concentrations of acetone and observed using a transmission electron microscopy (Zeiss, Oberkocken, Germany). The investigation of the hyperthermic properties of MS was performed by recording the temperature kinetics of the sample dispersed in a gel matrix (agarose at 0.25% w/w) during the exposition to an alternate magnetic field (183 kHz and 17 kA/m).

Results

TEM images reported in Fig.1 (a,b) show that MS are octahedral crystals organized in chains of about 20 nanoparticles. The length of chains depend on the parameters selected for bacterial culture. The size of iron core in MS was 42±9 nm. The transversal relaxivity of MS, measured in agarose gel (0.25%) at 4.7 T by using imaging techniques was comparable to the relaxivity of Endorem. T2w Images of HT-29 xenografts in which MS (1 mg of extracted MS) were injected are shown in Fig. 1(c,d): the presence of MS is well detectable as a dark region thanks to high transversal relaxivity. The values of transversal relaxivity in aqueous solution (Fig.1e) are similar to or higher than the ones of Endorem, in the whole investigated frequency range . Due to the dimensions of magnetite crystals inside the MS chains, the system exhibits a superparamagnetic behavior, probably very close to the ferromagnetic region transition. This is demonstrated by the hysteresis loop at room temperature (300 K), where coercivity, even if it is non-zero, stays at very low value (Hc = 30 Oe). Furthermore, in ZFC/FC investigation, a superparamagnetic blocking temperature around 300 K can be observed (Fig.1f). Finally, preliminary results indicate that MS have a high hyperthermic efficiency. In fact a Specific Absorption Rate (SAR) of 806 ± 85 W per grams of iron, in line with data reported in the literature, was measured.

Discussion

In this paper several techniques have been used to characterize magnetic nanoparticles naturally produced by the magnetotactic bacteria *Magnetospirillum gryphiswaldence.* Thanks to high transversal relaxivity, these nanoparticles are efficient contrast agents for MRI. Moreover they are characterized by superparamagnetic behavior (close to the ferromagnetic transition) and high hyperthermic efficiency. The last property makes these nanoparticles potential therapeutic agents.

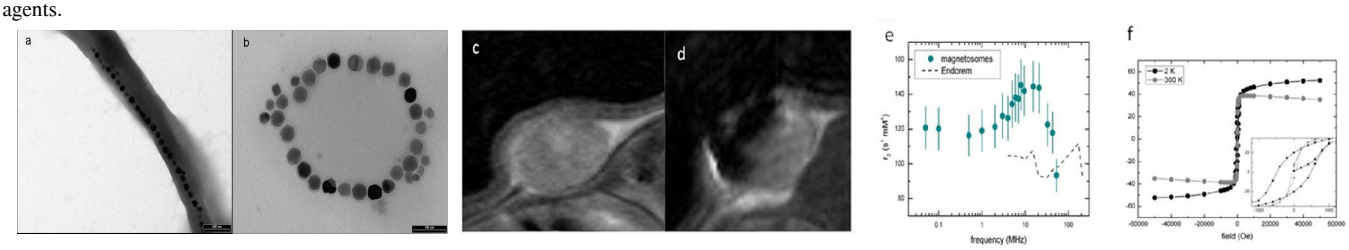

Fig.1 TEM images of MS . a) organization of MS in chains in the bacteria (scale bar 200 nm). b) Typical conformation of chains is maintained after MS isolation (scale bar 100 nm. c,d) MRI images of tumor before and 24h after to MS injection, e) NMR dispersion profile: transverse relaxivity as a function of proton Larmor frequency. f) Hysteresis loops of a freeze-dried magnetosomes sample at high (300K, gray symbol) and low (2K black symbols) temperature. In the inset the enlargement of the low field part of the loops is shown.

Conclusion

Iron-oxide nanoparticle naturally produced by MTB have been exploited for applications as theranostic agents. Thanks to high transversal relaxivity and high hyperthermal efficiency, MS are potential contrast and therapeutic agents.

Reference

1.Blakemore R. Magnetotactic bacteria. Science 1975; 190(4212):377-9

2.Grunberg K, Muller EC, Otto A, et al. Biomedical and proteomic analysis of the MN membrane in Magnetospirillum gryphiswaldense. Appl Environ Microbiol. 2004;70(2):1040-50