FePt Nanoparticles as Promising Magnetic Nanobeads for Biomedical Applications

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Abstract

L10-phase iron-platinum (FePt) nanoparticles (NPs) are an excellent magnetic material which is expected to be utilized for ultra-high density magnetic storage media because of their superior magnetic properties. Meanwhile, fcc-phase FePt NPs are also expected to be a high-performance nanomagnet for magnetic medicine, including magnetic hyperthermia, magnetic resonance imaging, magnetic cell/protein/organelle separation and magnetofection, because they present a superparamagnetic behavior with high saturation magnetization and high chemical stability. We outline the effectiveness of fcc-phase FePt NPs for some medical applications in comparison to superparamagnetic iron oxide (SPIO) NPs. In addition, some recent developments regarding magnetic-fluorescent and magnetic-plasmonic dual functional core-shell NPs are presented.

Key Words: FePt, Iron platinum, Superparamagnetism, Magnetic medicine

I. INTRODUCTION

Superparamagnetic iron oxides (SPIOs) including γ-Fe2O3 (maghemite) and Fe3O4 (magnetite) nanoparticles (NPs) are biocompatible and relatively easy to synthesize; these properties make them the most used magnetic NPs in biomedicine to date. They have been used for several decades and have contributed to both diagnostics such as magnetic resonance imaging (MRI) contrast agents and therapeutics such as magnetic hyperthermia. However, the relatively low saturation magnetisation ($M_s$) of SPIOs (~300–400 emu·cm$^{-3}$) limits their potential in these applications. The response of SPIO NPs to an external magnetic field may be sufficient for imaging purposes to some extent, but their suitability in magnetically targeted drug delivery is doubtful, namely because they can not effectively be directed within the human body by magnetic forces because the saturation magnetization is too low. Enhancement of the magnetic moment of magnetic NPs is key for improvement of many applications in biomedicine.

Considering the characteristic size of biological systems (10–100 μm for a cell, 20–450 nm for a virus, 5–50 nm for a protein and 2 nm in width and 10–100 nm in length for a gene), magnetic NPs with smaller dimensions than normally used SPIO NPs are preferred as they would increase the spatial resolution. Using magnetic NPs, which have higher saturation magnetization and higher magnetocrystalline anisotropy energy than SPIOs, one can significantly improve efficiency in various biomedical applications. Moreover, these magnetically superior ultrasmall magnetic NPs could lead to revolutionary and novel clinical applications.

Recently, mono- and bimetallic superparamagnetic NPs have become readily available thanks to the development of a range of synthetic techniques. In general, the metallic magnetic NPs exhibit higher magnetic properties than oxide magnetic NPs. For example, elemental Fe and Co have $M_s$ of about 1700 and 1400 emu·cm$^{-3}$, respectively. Bimetallic FePt, CoPt, FeCo and SmCo5 alloys each have $M_s$ of about 1000, 800, 1900 and 900 emu·cm$^{-3}$, respectively. Note that iron oxides have a $M_s$ of about 400~500 emu·cm$^{-3}$. With higher $M_s$, magnetic NPs experience higher driving forces under a magnetic field, and thus, the efficacy of drug delivery or magnetic separation will be greatly improved. In the case of hyperthermia therapy, the optimal size of magnetic NPs as heating elements varies depending on the magnetocrystalline anisotropy energy. Roughly speaking, the optimal magnetic NP size for hyperthermia is inversely proportional to the magnetocrystalline anisotropy constant. On the other hand, the heating rate increases with increasing $M_s$. In the case of FePt, the magnetocrystalline anisotropy energy (206 kJ·m$^{-3}$) is much higher than that of the various iron oxides (5~10 kJ·m$^{-3}$), and thus, one can reduce the optimal magnetic NP size. In general, for biological applications, the smaller the NPs the better if the performance is equal. For these reasons, the next generation of magnetic NPs increasingly attracts attention in various biomedical fields [1]. We consider SPIO NPs to be a classical type of magnetic NP.

In this paper, we review the effectiveness of FePt superparamagnetic NPs for some medical applications in comparison to SPIO NPs [1–5]. In the case of the most popular synthetic method of FePt NPs in which Fe(CO)$_3$ is used as a precursor, in addition to the drawbacks of high toxicity and high flammability, the atomic composition distribution of the final NPs...
is extremely broad. Because the magnetic properties of FePt NPs are strongly dependent not only on the size but also on the composition, we have developed a synthetic method which can obtain monodispersed equiatomic FePt NPs with a narrow composition distribution [6,7]. This increases the potential for putting FePt NPs to practical use in biomedical fields at an accelerated rate. In addition, some recent developments regarding heterostructured NPs which have FePt NPs as a core and semiconductor or plasmonic materials as a shell, for example, magnetic-fluorescent FePt@CdSe [4] and magnetic-plasmonic FePt@Ag core-shell NPs, will be briefly discussed [5]. These dual functional NPs are expected to be highly promising magnetic nanoprobes, nanotracers, nanocarriers or nanoheaters and will be very useful for many biomedical applications.

II. EFFECTIVENESS OF FEPT NPS AS MRI CONTRAST AGENTS

MRI enables one to obtain a cross-sectional view of a human body noninvasively by utilizing the nuclear magnetic resonance of protons that comprise a large percentage of the body. When a human body is placed in a large magnetic field, many of the free hydrogen nuclei align themselves with the direction of the magnetic field (Larmor precession). In this situation, a radiofrequency (RF) pulse with a frequency equal to the Larmor frequency is applied perpendicular to the magnetic field. This RF pulse causes the net magnetic moment of the nuclei to tilt away from the magnetic field. Once the RF pulse is removed, the nuclei return to their equilibrium state, such that the net magnetic moment of the nuclei is again parallel to the magnetic field (relaxation). During relaxation, the nuclei lose energy by emitting a so-called free-induction decay (FID) response signal. The FID response signal is measured by a conductive field coil and is reconstructed to obtain MR images. There exist the following two relaxation processes: longitudinal (spin-lattice) and transverse (spin-spin) relaxation processes. The time constants of these relaxation processes are known as $T_1$ and $T_2$, respectively.

SPIO NPs have been widely used as MRI $T_2$-shortening agents in clinical practice, and have played an important role in the detection of small metastatic liver tumors and/or hepatocarcinomas. Internally administered SPIO contrast agents are selectively ingested by Kupffer cells inside the liver. Due to their magnetic susceptibility effect, $T_2$ is significantly shortened; thus, the signal is reduced in the proton density-weighted image or the $T_2$-weighted image. Therefore, the signal intensity from normal hepatic tissue decreases. This causes the contrast enhancement of malignant metastatic liver tumors or hepatocarcinomas, which do not contain Kupffer cells. The detection capability of SPIO-enhanced MRI is considered to be almost equal to that of computed tomography during arterial portography (CTAP), which is thought to be the most sensitive diagnostic imaging technique for the detection and localization of metastatic liver cancer. In addition, SPIO-enhanced MRI can be used in patients who are unable to undergo CTAP due to, for example, iodine allergy or renal insufficiency.

![Fig. 1](image-url) Inverse longitudinal and transverse relaxation times: (a) $T_1^{-1} - T_1(0)^{-1}$ and (b) $T_2^{-1} - T_2(0)^{-1}$ for TMAOH-capped FePt NPs dispersed in pure water (open circles and dashed line) and in a 5-wt% TMAOH solution (filled circles and solid line) as a function of NP concentration, C.

An enhancement of the $T_2$-shortening effect and an improvement in molecular recognition ability can be expected when
one uses FePt NPs as MRI contrast agents instead of conventional SPIO NPs because of their higher $M_s$ than SPIO NPs. We evaluated the $T_1$- and $T_2$-shortening abilities of FePt NPs by using inversion-recovery spin-echo (IRSE) and Carr–Purcell–Meiboom–Gill (CPMG) pulse sequences, respectively [2]. The $T_1$ and $T_2$ values were obtained from FePt NPs dispersed in pure water and from FePt NPs dispersed in a 5-wt% tetramethylammonium hydroxide (TMAOH) aqueous solution. As shown in Fig. 1, the inverse relaxation times were almost linearly proportional to the FePt NP concentration according to the following equation:

$$R_{1/2}C = T_{1/2}(C)^{-1} - T_{1/2}(0)^{-1}. \quad (1)$$

where $C$ is the NP concentration; $R_1$ and $R_2$ are the longitudinal and transverse relaxivities, respectively. $T_1(0)$ and $T_2(0)$ are the proton relaxation times in pure solvents. Consequently, the $R_1$ and $R_2$ values of FePt NPs dispersed in pure water were found to be 7.4 and 239 s$^{-1}$·mM$^{-1}$, respectively. On the other hand, the $R_1$ and $R_2$ values of FePt NPs dispersed in a 5wt% TMAOH aqueous solution were 9.8 and 327 s$^{-1}$·mM$^{-1}$, respectively. Hence, the $R_2/R_1$ relaxivity ratio of TMAOH-capped FePt NPs was found to be 32–33. The higher the $R_2/R_1$ relaxivity ratio the higher the $T_2$ effect and the signal decrease on $T_2$-weighted images. Ferucarbotran (Resovist®, Schering AG, Berlin, Germany) is a carboxydextran-coated SPIO with a high relaxivity ratio $R_2/R_1 = 9.6$ ($R_1 = 19.4$ s$^{-1}$·mM$^{-1}$ and $R_2 = 185.8$ s$^{-1}$·mM$^{-1}$), which has been widely used in clinical practice as a liver-specific MRI contrast agent. Ferumoxide (Feridex®, Advanced Magnetics, Cambridge, USA) is a dextran-coated SPIO with the relaxivity ratio $R_2/R_1 = 4.0$ ($R_1 = 40$ s$^{-1}$·mM$^{-1}$ and $R_2 = 160$ s$^{-1}$·mM$^{-1}$). Ferumoxtran (Combidx®, Advanced Magnetics, Cambridge, USA) is a dextran-coated ultrasmall superparamagnetic iron oxide (USPIO) used as a lymph node-specific contrast agent. The relaxivity ratio of ferumoxtran is $R_2/R_1 = 2.3$ ($R_1 = 23$ s$^{-1}$·mM$^{-1}$ and $R_2 = 53$ s$^{-1}$·mM$^{-1}$). FePt NPs can be seen to have a superior $T_2$-shortening ability than other existing SPIO-based MRI contrast agents, such as Resovist®, Feridex® and Combidx® [2].

### III. EFFECTIVENESS OF FEPT NPS AS HEATING ELEMENTS FOR MAGNETIC HYPERThERMIA

Hyperthermia is one among many techniques used in oncology, based on heating tumors for therapeutic purposes, and is usually used as an additive therapy with standard treatments, such as radiotherapy and chemotherapy. The basic principle of cancer treatment is to eliminate only cancerous cells, distinguishing them from normal cells. They are distinguished by visual inspection in surgical procedures. However, cellular discrimination is difficult. Although various efforts have been made to attain tumor-selective radiotherapy and chemotherapy, there remain considerable challenges in these techniques. Hyperthermia is superior to other therapeutic techniques on this point. The blood flow is insufficient in tumors and the inadequate blood flow makes tumors more acidic due to the lactic acid buildup in the tumor tissues from lack of oxygen. In general, cells die easily when the environment becomes acidic, because the temperature-sensitivity of the cell increases. Moreover, cancer cells have a lower thermal resistance than normal cells. In consequence, one can eliminate cancer cells selectively by rising the local temperature at the site of the tumor.

The magneto-thermo-cytolysis (or the magneto-thermoablation) is a promising technique thanks to the development of precise methods for synthesizing functionalized magnetic NPs. Magnetic NPs with functionalized surfaces, which have high specificity to a tumor tissue, are used as heating elements for hyperthermia. The process involved in the magnetic hyperthermia is based on the energy dissipation when a ferromagnetic material is placed on an external alternating magnetic field. The energy dissipation of magnetic NPs consists of the following two effects: the Néel relaxation and the Brownian relaxation. For efficient magnetic hyperthermia, the magnetic NPs are required to have a high saturation magnetization in order to minimize the doses needed for temperature increase. In this context, FePt NPs are promising nanoheaters because they possess a high $M_s$, high magnetocrystalline anisotropy energy, and high chemical stability. We theoretically evaluated the applicability of FePt NPs for magnetic hyperthermia, comparing them to $\gamma$-$Fe_2O_3$ (maghemite), Fe$_3$O$_4$ (magnetite) and FeCo (permendur) NPs [3].

Fig. 2 shows comparative heating rates for aqueous monodispersions of the various magnetic NPs. The amplitude and frequency of the applied magnetic field was fixed at 50 mT and 300 kHz. The carrier liquid is pure water in all cases. With these conditions, fcc-phase FePt NPs yield the largest heating rates in the size range of $D < 25$ nm ($D$: mean diameter). The operative size of each NP system, $D_{\text{max}}$, for the maximum heating rate, is 9 nm for FePt NPs, 19 nm for...
magnetite, 23.5 nm for maghemite, and 34 nm for FeCo NPs. The FeCo NPs have the highest heating rate as shown in Fig. 2. The maghemite NPs also have a large heating rate as well as magnetite NPs. However, the size ranges of FeCo and maghemite, where the heating is possible, are much larger than the typical size ranges of standard ferrofluids (D = 8-10 nm). In general, the colloidal stability and the molecular recognition property of magnetic NPs becomes impaired when ca. D > 20 nm. In consequence, FePt NPs are found to be promising heating elements for magnetic hyperthermia compared to other magnetic NPs such as maghemite, magnetite, and FeCo NPs [3].

IV. MAGNETIC-FLUORESCENT FEPT@CDSE CORE-SHELL NPS
Magnetic-fluorescent hybrid materials composed of magnetic NPs and semiconductor quantum dots (QDs) in novel heteronanostructures are intriguing because they can lead to new bioapplications. In the case of the NPs discussed here the magnetic core particle responds to an external magnetic field allowing protein separation, targeted drug delivery or other magnetic based applications while the QD shell is useful in cell labeling, cell migration tracking, in vivo imaging, etc. The combination of these two materials allows multiplexing of techniques or even new bioanalysis schemes to be employed.

The synthesis of core-shell structures with uniform structural parameters however is challenging. In this case, the NPs were created in a multi-step organic based wet chemical approach. Recently, we developed a new direct synthetic technique of well-defined FePt@CdSe core-shell NPs [4]. The resulting NPs appeared uniform in traditional TEM analysis, but to gain an understanding of the fine structural properties, we turned to the scanning TEM (STEM, JEOL JEM-ARM200F) instrument. High angle annular dark field (HAADF) - STEM imaging and EDS elemental mapping of individual NPs was performed to confirm the FePt@CdSe core-shell structure. Fig. 3 shows the STEM image of a single NP containing relatively heavy elements at the core and lighter elements in the shell. The result clearly elucidates the FePt@CdSe core-shell structure where the FePt core is more dense than the CdSe shell.

The blocking temperature (T_B) was addressed using zero-field-cooled (ZFC) and field-cooled (FC) measurements. The temperature sweeps were collected under a constant field of 500 Oe. The magnetization (M-H) curve was measured as a function of the applied field at temperatures over T_B. Fig. 4 shows temperature dependence of magnetization (FC and ZFC curves) and field dependence of magnetization (M-H curves) at 5 K for 4.2-nm bare FePt NPs and FePt@CdSe NPs. The blocking temperatures were almost the same for FePt NPs and FePt@CdSe NPs, and is approximately 55 K. The magnetocrystalline anisotropy constant (K) was estimated using the following equation:

\[ K = \frac{\ln(t_{ico}/t_0)k_BT_B}{V}. \]
where \( t_{\text{meas}} = 30 \, \text{s} \), \( \tau_0 = 10^{10} \, \text{s} \), \( k_B \) and \( V = 3.88 \times 10^{-26} \, \text{m}^3 \) are the measurement time, the relaxation time, the Boltzmann constant and the volume of a single NP, respectively. \( K \) was calculated to be 472 kJ·m\(^{-3}\) for both FePt and FePt@CdSe NPs. Coercivity of FePt@CdSe NPs is about 200 Oe, half the value of the FePt NPs (400 Oe). FePt-based \( M_S \) of FePt@CdSe NPs was estimated to be 23 emu·g\(^{-1}\), which is higher than that of the FePt NPs (19 emu·g\(^{-1}\)). According to the Stoner-Wohlfarth theory, the coercivity of NPs is determined by \( K \) and \( M_S \):

\[
H_C = \frac{2K}{\mu_0 M_S}. \tag{3}
\]

where \( \mu_0 \) is the universal constant of permeability in free space. This may explain why the coercivity of FePt@CdSe NPs is smaller than that of FePt NPs. The enhanced \( M_S \) of FePt@CdSe can be a result of the passivation of the surface of FePt NPs by the CdSe shell. However, this cannot completely explain why the coercivity of FePt@CdSe NPs is just half that of FePt NPs. Though there should be another reason for the significant reduction in the coercivity, it is not clear at this moment. The formation of CdSe shell could reduce a nonmagnetic shell (surface dead layer), which is formed by the interaction of organic ligands to the surface of FePt NPs, and/or a canted spin layer due to broken symmetry at the surface [4].

V. MAGNETIC-PLASMONIC FePt@Ag CORE-SHELL NPS

Plasmonic (especially Au and Ag) NPs are ideal candidates for various sensing/imaging applications—including biological sensors, cellular imaging, immunodiagnosis, and DNA sequencing—because of their excellent localized surface plasmon resonance (LSPR) and surface enhanced Raman scattering (SERS) properties. The combination of plasmonic and magnetic materials in a single nanostructure has the potential to open up new applications in biology, such as immunomagnetic separation under plasmonic imaging monitoring, dual mode imaging (MRI and plasmonic imaging), and SERS sensing. There have been a number of reports on the combination of magnetic NPs with plasmonic materials for different purposes. Among those magnetic-plasmonic NPs, FePt@Ag NPs seem to be better suited for biological sensing/imaging applications when compared with other magnetic-plasmonic NPs, in terms of their size (less than 20 nm), magnetic properties, and optical properties; these properties result from the high \( M_S \) of FePt NPs, and the extinction coefficient and SERS activity of Ag NPs, which are the highest of any of the plasmonic NPs.

In view of this, we developed a scheme for the synthesis of FePt@Ag NPs with high SERS activity, high colloidal stability, and good magnetic separation capabilities, with the aim of using the NPs for biological sensing/imaging applications [5]. Fig. 5 shows the UV-Vis spectra for Ag, FePt and FePt@Ag\(x\) NPs where the subscript \( x \) denotes the Ag shell thickness in nanometers. The mean sizes of the Ag and FePt NPs were 15.8 ± 3.0 and 4.5 ± 0.5 nm. The Ag and FePt@Ag\(x\) NPs exhibited a LSPR peak in the visible region, while the FePt NPs showed only a broad absorption profile. The LSPR peak wavelengths for Ag, FePt@Ag\(x\), and FePt@Ag\(x\) NPs were 399, 397, and 412 nm, respectively. It is noteworthy that the LSPR peak for the FePt@Ag\(x\) NPs was slightly red-shifted compared with that for the Ag NPs.

The colloidal stability of the FePt@Ag NPs was evaluated by measuring the size distribution of the NPs in hexane, using DLS. The FePt@Ag\(x\) NP dispersion was stored under ambient laboratory conditions for 2.5 months, and the
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Recent advancements in our own research regarding superparamagnetic FePt NPs have been reviewed. FePt NPs exhibit superior magnetic properties compared to conventional SPIOs, and thus, are expected to be highly utilized in various biomedical applications such as removal of blood-borne toxins in the body, magnetic separation of cells/proteins, magnetic immunoassay, magnetic gene transfection, magnetic cell targeting for cell therapies, magnetic drug delivery, hyperthermia cancer treatment and magnetic particle imaging. The combination of magnetic FePt components with other optically active components in a nanoheterostructure leads to multifunctionality, opening a new window for biomedical applications.

VI. SUMMARY

Fig. 5 UV-Vis spectra for FePt (black), Ag (red), FePt@Ag3.2 (green), and FePt@Ag5.7 (blue). The inset shows a photograph of the diluted suspensions of FePt (A) and FePt@Ag5.7 (B) NPs.

Fig. 6 Photographs of FePt@Ag5.7 NPs in hexane without (A) and with magnet (B).

Fig. 7 SERS spectra of TP obtained using (A) FePt NPs, (B) Ag NPs, (C) FePt@Ag5.7 NPs (droplet deposition method), and (D) FePt@Ag5.7 (magnetic deposition method).
applications including immunomagnetic separation under fluorescent/plasmonic imaging monitoring, dual mode imaging (MRI and fluorescent/plasmonic imaging), and SERS sensing.

REFERENCES