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Monodisperse Magnetic Nanoparticles for Theranostic Applications

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Conspectus

The development of highly effective medicine requires the on-time monitoring of the medical treatment process. This combination of monitoring and therapeutics allows a large degree of control on the treatment efficacy and is now commonly referred to as “theranostics”. Magnetic nanoparticles (NPs) provide a unique nano-platform for theranostic applications due to their comparable sizes with various functional biomolecules, their biocompatibility and their responses to the external magnetic field. Recent efforts in studying magnetic NPs for both imaging and therapeutic applications have led to great advances in NP fabrication with controls in dimension, surface functionalization and magnetic property. These magnetic NPs have been proven to be robust agents that can be target-specific for enhancing magnetic resonance imaging sensitivity and magnetic heating efficiency. These, plus the deep tissue penetration of magnetic field, make magnetic NPs the most promising candidates for successful theranostics in the future.

In this Account, we review the recent advances in the synthesis of magnetic NPs of iron oxide, Fe, as well as FePt and FeCo NPs for imaging and therapeutic applications. We will first introduce briefly nanomagnetism, magnetic resonance imaging (MRI), and magnetic fluid hyperthermia (MFH). We will then focus on chemical synthesis of monodisperse magnetic NPs with controlled sizes, morphologies, and magnetic properties. Typical examples in using monodisperse magnetic NPs for MRI and MFH are highlighted.

1. Introduction

The development of highly effective medicine requires continuous and timely monitoring of the medical treatment process. This combination of monitoring (also called imaging or diagnostics) and therapeutics allows a large degree of control on the treatment efficacy in

different individuals and is now commonly referred to as “theranostics”.¹ Due to the presence of large percentage of surface atoms, inorganic nanoparticles (NPs), especially those in the dimension < 20 nm, have unique physical and chemical properties that are not observed in their bulk forms.² This, plus their comparable sizes to biomolecules, makes inorganic NPs ideal functional probes for simultaneous imaging and therapeutic (theranostic) applications. Among various NPs studied thus far, magnetic NPs containing ferromagnetic iron (Fe) and cobalt (Co) as well as their alloys and oxides have been proven to be the most promising probes for theranostics with the desired imaging sensitivity and therapeutic efficacy.^{3–6}

A ferromagnetic NP at the size smaller than 20 nm often contains a single magnetic domain with one collective magnetization direction (Figure 1A).⁷ Once these NPs are placed in an external magnetic field (H), their magnetization directions can be aligned along the field direction to achieve magnetic saturation with overall magnetization reaching M_s (Figure 1B&C). The ease at which a NP can be aligned is measured by its susceptibility (M/H). Reduction of the field strength leads to a certain degree of decrease in M due to magnetic relaxations but NPs tend to retain their magnetization direction and have a remnant magnetization (M_r) at zero field strength. To demagnetize these NPs, the external magnetic field must be applied in the opposite direction. The field strength required to demagnetize these NPs is defined as coercivity (H_c) (Figure 1C). When the size of a ferromagnetic NP is reduced to a level where the thermal energy is comparable to the magnetic anisotropy energy, this NP is magnetically unstable and is said to be superparamagnetic.⁸ A group of superparamagnetic NPs can be easily magnetized (with large susceptibility) to reach M_s , but they have no H_c and M_r (Figure 1D). Magnetic NPs in superparamagnetic state have much weaker magnetic dipole interactions and therefore are readily stabilized and dispersed in liquid media.

The diagnostic applications of magnetic NPs are realized in magnetic resonance imaging (MRI).⁹ The technique is based on the difference in nuclear magnetic relaxations of the water protons in biological solutions and around solid tissues. A contrast agent modifies the nuclear magnetic relaxation rate of its surrounding protons and changes the signal contrast. Its contrast enhancement effect is measured by the relaxation rate, $R = 1/T$ (s^{-1}) and the relaxivity, $r = R/\text{concentration}$ ($\text{mM}^{-1}\cdot\text{s}^{-1}$). The higher relaxivity corresponds to a better contrast effect. Complexes of paramagnetic metal ions (such as Gd^{3+}) serve as a T_1 contrast agent¹⁰ while magnetic NPs are generally used as a T_2 contrast agent. Their relaxation rate and relaxivity are dependent on $(M_s V)^2$ and d^{-6} with V being the NP volume and d the distance between the magnetic core and the surrounding protons.¹¹ To have better contrast effect, magnetic NPs should have high magnetization (M_s), large volume (V) and thin coating (small d). However, for biological imaging applications, hydrodynamic sizes of a NP is better controlled to be below 50 nm in order for NPs to have long circulation time and to avoid non-specific uptake.¹² Therefore, magnetic NPs with high magnetization and thin coating are often pursued for sensitive MRI.

Under a fast switching magnetic field, a group of superparamagnetic NPs can become ferromagnetic with their magnetization direction switching quickly along the field directions. The frictions caused by the physical rotation of a NP, Brownian relaxation, and the magnetization reversal within the NP, Neel relaxation, lead to the loss of magnetic energy and the generation of thermal energy. The heating power of these NPs is directly related to $A \cdot f$ where A is the ferromagnetic hysteresis area and f the frequency of the alternating magnetic field.¹³ Used for cancer therapy, this magnetic heating technique has long been known as magnetic fluid hyperthermia (MFH).^{14,15} To maximize the NP heating power, the hysteresis area A must be as large as possible. However, hyperthermia limitations require that the product $H_{\text{max}} \cdot f$ should be below $5 \times 10^9 \text{ A}\cdot\text{m}^{-1}\cdot\text{s}^{-1}$ with f being above 50

kHz.¹⁴ To ensure optimum MFH effect under the common hyperthermia conditions, magnetic NPs should have small H_c , large susceptibility and high M_s . The NP heating efficiency is measured by the specific absorption rate (SAR, $\text{W}\cdot\text{g}^{-1}$). For practical therapeutic applications with minimized side effects, it is critically important to obtain optimum heating efficiency to reach the desired hyperthermia temperature at 41–46°C, not thermoablation at greater than 50°C.

2. Chemical Synthesis of Magnetic NPs

To succeed in theranostic applications, magnetic NPs should first be made monodisperse. They should have high magnetization and large susceptibility. They should also be small with a hydrodynamic size < 50 nm to have extravasation ability and to be stable against uptake by the reticuloendothelial system (RES).¹⁶ Among various magnetic NPs studied, iron oxide, Fe, as well as FeCo and FePt NPs are especially attractive for imaging and therapeutic applications.

2.1. Iron Oxide NPs

Magnetite (Fe_3O_4) and ferrite MFe_2O_4 NPs ($\text{M} = \text{Mn}, \text{Zn}$) NPs are often selected for biomedical applications due to their chemical/magnetic stability and low cytotoxicity.¹⁷ In the bulk form, these materials are ferrimagnetic due to the antiferromagnetic coupling among Fe(III) in their inverse spinel structure.¹⁸ However, this antiferromagnetic coupling may be eliminated in nanoscale as demonstrated recently by higher magnetizations observed in the ZnFe_2O_4 NPs.¹⁹ In general, iron oxide NPs smaller than 20 nm are superparamagnetic at room temperature and have mass magnetizations below their room temperature bulk values at 92 emu/g for Fe_3O_4 and 80 emu/g for MnFe_2O_4 .

Monodisperse Fe_3O_4 NPs are often made by reductive decomposition of metal acetylacetonate (acac) in a high temperature organic solution phase.^{20–22} In one synthesis, $\text{Fe}(\text{acac})_3$ is dissolved in diphenyl ether in the presence of 1,2-hexadecanediol, oleic acid and oleylamine (Figure 2A). 1,2-hexadecanediol functions as an organic reducing agent to facilitate the formation of Fe_3O_4 . Oleic acid and oleylamine are used as surfactants. The step heating at 200 °C and 265 °C are applied to control Fe_3O_4 nucleation at 200 °C and growth at 265 °C. This synthesis gives 4 nm Fe_3O_4 NPs. With benzyl ether as a solvent and heating temperature raised 300 °C, 6 nm Fe_3O_4 NPs are obtained. The small Fe_3O_4 NPs can serve as seeds and larger Fe_3O_4 NPs can be made via the seed-mediated growth of Fe_3O_4 in the reductive decomposition of $\text{Fe}(\text{acac})_3$. Figure 2B shows the typical TEM image of an assembly of 16 nm Fe_3O_4 NPs. Hysteresis loops of the 16 nm Fe_3O_4 NPs measured at both 10 K and at room temperature show that the NPs are ferrimagnetic at 10 K ($H_c = 450$ Oe) and superparamagnetic at 300 K ($M_s = 83$ emu/g) (Figure 2C).

Fe oleate is another promising precursor used for the synthesis of iron oxide NPs.²³ In this approach, Fe-oleate is first prepared from iron chloride ($\text{FeCl}_3\cdot 6\text{H}_2\text{O}$) and sodium oleate in a mixture solvent of ethanol, water and hexane at 70 °C. The resulting Fe-oleate complex is then dissolved in a high-boiling-point solvent and then heated to 300 °C to produce monodisperse iron oxide NPs with oleate serving as a surfactant. The synthesis provides a reliable way of making large amount (up to 40 g) of monodisperse NPs with excellent control on NP sizes (tunable from 5 to 22 nm). Monodisperse iron oxide NPs can also be prepared by thermal decomposition of $\text{Fe}(\text{CO})_5$ followed by oxidation.²⁴ By combining decomposition/oxidation of $\text{Fe}(\text{CO})_5$ and reductive decomposition of iron oleate complex, the iron oxide NPs can be made only 1 nm difference in diameter.²⁵ Recently, ultra-small Fe_3O_4 NPs ranging from 2.5 nm to 5 nm are made by thermal decomposition of $\text{Fe}(\text{CO})_5$ in benzyl ether at 300 °C followed by room temperature air oxidation.²⁶ Similar to that in the synthesis of Fe_3O_4 NPs, the high temperature reaction of $\text{Fe}(\text{acac})_3$ and $\text{Mn}(\text{acac})_2$ gives

monodisperse MnFe_2O_4 with their size, composition and shape controlled by the reactant concentrations.²⁷

Thermal decomposition of $\text{Fe}(\text{CO})_5$ followed by controlled oxidation has been applied to make hollow or porous hollow Fe_3O_4 NPs.^{28,29} In this synthesis, amorphous Fe NPs are first made via the decomposition of $\text{Fe}(\text{CO})_5$ in the presence of oleylamine. The Fe NPs are exposed to air briefly to form core/shell Fe/ Fe_3O_4 NPs with both Fe and Fe_3O_4 in the amorphous state. Controlled oxidation of these core/shell NPs at high temperature ($> 200^\circ\text{C}$) by trimethylamine N-oxide (Me_3NO) yields hollow Fe_3O_4 NPs. In the hollow structure, the Fe_3O_4 shell is polycrystalline. Upon further heating, the Fe_3O_4 crystalline domain is increased, leading to the formation of porous hollow Fe_3O_4 NPs.

2.2. Au- Fe_3O_4 NPs

Dumbbell-like Au- Fe_3O_4 NPs are introduced as one class of multifunctional NPs to control the conjugation of different targeting agents and therapeutic drugs to the NP surface.^{30–32} The Au- Fe_3O_4 NPs are prepared via the decomposition of $\text{Fe}(\text{CO})_5$ over the surface of the pre-formed Au NPs followed by oxidation in air, as illustrated in Figure 3A. The Au NPs can be either synthesized in situ by injecting HAuCl_4 solution into the reaction mixture or pre-made in the presence of oleylamine. More recently, the Au seeding NPs are made by the reduction of $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$ with *tert*-butylamine borane complex (TBAB) in 1,2,3,4-tetrahydronaphthalene (tetralin) and oleylamine.^{33,34} To synthesize Au- Fe_3O_4 NPs, $\text{Fe}(\text{CO})_5$ is injected into 1-octadecene solution containing the Au seeds. Fe nucleates and grows onto Au NPs. Upon exposure to air, the Fe NPs are oxidized to Fe_3O_4 NPs, giving Au- Fe_3O_4 NPs. The size of the Fe_3O_4 NPs is controlled by adjusting the ratio between $\text{Fe}(\text{CO})_5$ and Au. Figure 3B&C show the TEM images of the 6 nm Au seeding NPs and the 6–17 nm Fe_3O_4 NPs. Like Fe_3O_4 NPs, the Au- Fe_3O_4 NPs are also superparamagnetic at room temperature.

2.3. Metallic Fe NPs

Metallic Fe is a typical class of ferromagnetic materials with $M_s = 218 \text{ emu/g Fe}$. Magnetic NPs of Fe with high magnetizations are important for sensitive MRI and MFH applications. However, metallic Fe NPs are chemically unstable and are oxidized easily to form various iron oxide NPs with much reduced magnetizations.³⁵ Therefore one of the biggest challenges in the synthesis is how to stabilize these NPs against fast oxidation.

The common approach to monodisperse Fe NPs is via thermal decomposition of organometallic precursors.^{35,36} In a recent synthetic demonstration, monodisperse Fe NPs are made by thermal decomposition of $\text{Fe}(\text{CO})_5$ in 1-octadecene and oleylamine.³⁷ To stabilize these Fe NPs, a dense shell of the crystallized Fe_3O_4 is created over the Fe core via controlled oxidation of Fe NPs by $(\text{CH}_3)_3\text{NO}$. The resultant Fe/ Fe_3O_4 NPs show the much enhanced stability after 24 h exposure to air. More recent synthesis shows that body centered cubic (bcc) Fe can be made by the decomposition of $\text{Fe}(\text{CO})_5$ in the presence of hexadecylammonium chloride.³⁸ These bcc-Fe NPs exhibit a drastically increased stability and magnetization and are promising for biomedical applications.

2.4. FePt and FeCo Alloy NPs

FePt and FeCo alloy NPs are two of the most interesting bimetallic NPs studied for biomedical applications. FePt NPs show the structure-dependent magnetic properties from strong ferromagnetism to superparamagnetism and FeCo NPs can offer high magnetization (FeCo has a bulk saturation magnetization of 240 emu/g). FePt NPs are chemically stable against oxidation but FeCo NPs are chemically unstable and require special treatment for long-term stabilization.

Thermal decomposition of $\text{Fe}(\text{CO})_5$ and reduction of $\text{Pt}(\text{acac})_2$ in the presence of 1,2-alkanediol is a common way for making monodisperse FePt NPs.^{39,40} The synthetic chemistry is illustrated in Figure 4A. Oleic acid and oleylamine are used for FePt NP stabilization. The composition of the FePt NPs is controlled by $\text{Fe}(\text{CO})_5/\text{Pt}(\text{acac})_2$ ratio. Figure 4B shows the TEM image of the 6 nm FePt NPs. Fine size tuning on FePt NPs between 2–5 nm is achieved by controlling the surfactant to metal ratio.⁴¹ Larger FePt NPs can be made by the decomposition of $\text{Fe}(\text{CO})_5$ and reduction of $\text{Pt}(\text{acac})_2$ without the presence of 1,2-alkanediol.^{42,43} In this synthesis, small Pt-rich FePt NPs are first formed from the reduction of $\text{Pt}(\text{acac})_2$ and the partial decomposition of $\text{Fe}(\text{CO})_5$ at temperature $< 200^\circ\text{C}$. More Fe atoms then coat on the existing Pt-rich FePt NPs, forming larger core/shell NPs. Heating the core/shell structure at 300°C leads to the Fe diffusion into the Pt-rich core and formation of FePt NPs. Figure 4C shows the TEM image of the 9 nm FePt NPs.

High moment FeCo NPs are synthesized by reductive thermal decomposition of $\text{Fe}(\text{CO})_5$ and $\text{Co}(\eta^3\text{-C}_8\text{H}_{13})(\eta^4\text{-C}_8\text{H}_{12})$ or $\text{Co}(\text{N}(\text{SiMe}_3)_2)_2$ in the presence of hexadecylamine and oleic acid,⁴⁴ or by interfacial diffusion between Fe and Co in the core/shell Co/Fe structure.⁴⁵ As-synthesized NPs have the mass magnetization of up to 200 emu/g. FeCo NPs may be better stabilized by a graphitic carbon made by methane chemical vapor deposition at 800°C .⁴⁶ These FeCo/graphitic carbon NPs have been proven to be stable and are promising for sensitive bioimaging applications.

3. Magnetic NPs as Contrast Agent for MRI Applications

Magnetic NPs with controlled magnetizations are promising contrast agents for sensitive MRI applications. This is demonstrated nicely by MFe_2O_4 NPs.¹⁹ These ferrite NPs (6–12 nm) have size-dependent magnetizations with 12 nm MnFe_2O_4 and Fe_3O_4 NPs showing the maximum mass magnetization values of 110 emu/g and 101 emu/g respectively. Once modified with 2,3-dimercaptosuccinic acid and placed in a 1.5 Tesla (T) field, these NPs show the T_2 -weighted MR images with the contrast consistent with their magnetization data – larger NPs with higher magnetization generate stronger contrast effect (Figure 5). Due to the thin molecular coating, these NPs have very large relaxivity values, $358\text{ mM}^{-1}\cdot\text{s}^{-1}$ for 12 nm MnFe_2O_4 NPs and $218\text{ mM}^{-1}\cdot\text{s}^{-1}$ for 12 nm Fe_3O_4 NPs.

The cancer detection sensitivity of the ferrite NPs has been evaluated. To perform the test, the NPs are coupled with the cancer-targeting Herceptin, a monoclonal antibody specifically binding to the HER2/neu marker over-expressed on the surface of breast and ovarian cancers.¹⁹ Cell lines with different levels of HER2/neu over-expression are chosen for the study. These are the Bx-PC-3, MDAMB-231, MCF-7 and NIH3T6.7 with their relative HER2/neu expression levels being 1, 3, 28 and 2300 respectively. The test on the cells incubated with MnFe_2O_4 -Herceptin indicates that the MR contrast enhancement is related directly to the HER2/neu expression level. In contrast, when stained with the Fe_3O_4 -Herceptin, only NIH3T6.7 cells are MR-detectable. Figure 6 shows the color coded MRI of a mouse implanted with the cancer cell line NIH3T6.7 and treated with 50 mg of NP-Herceptin. It can be seen that the tumor treated with the MnFe_2O_4 -Herceptin NPs show color changes from red to blue (Figure 6A–C) but those treated with the Fe_3O_4 -Herceptin NPs at the same dosage have no apparent color change (Figure 6D–F).

Ultra-small Fe_3O_4 NPs (< 10 nm in hydrodynamic diameter) are also tested by MRI for tumor-specific targeting. In this demonstration, the 4.5 nm Fe_3O_4 NPs are stabilized by 4-methylcatechol (4-MC).²⁶ A cyclic arginine-glycine-aspartic acid (cRGD) peptide, c(RGDyK), is coupled to 4-MC via the Mannich reaction (Figure 7A) for integrin targeting. Integrins are a family of cell adhesion molecules consisting of two non-covalently bound transmembrane subunits (α and β) and the integrin $\alpha_v\beta_3$, which binds to RGD-containing

components, is significantly upregulated on tumor vasculature and invasive tumor cells. The targeting ability of the c(RGDyK)-Fe₃O₄ NPs to integrin $\alpha_v\beta_3$ *in vivo* is evaluated by T₂-weighted MR imaging with mice bearing U87MG tumors. The r_2 relaxivity of the c(RGDyK)-Fe₃O₄ NPs is measured to be 165 mM⁻¹•s⁻¹, which is larger than that of the commercial Feridex NPs (104 mM⁻¹•s⁻¹) with the similar core size. The tumor MR signal intensity decreases by 40% after the injection of c(RGDyK)-Fe₃O₄ NP dispersion (Figure 7B&C).

Dumbbell-like Au-Fe₃O₄ NPs have been studied as probes for multimodality imaging applications.³² In this structure, Au NPs are optically active and Fe₃O₄ NPs are magnetically active. The presence of two different NP surfaces within one nanostructure facilitates the controlled functionalization of each NP. Figure 8A illustrates the surface functionalization of the Au-Fe₃O₄ NPs. The original oleate/oleylamine coating on Fe₃O₄ is replaced by a catechol unit present in the dopamine molecule that is linked to PEG, and oleylamine around Au is exchanged by HS-PEG-NH₂ with HS- attaching to Au. The epidermal growth factor receptor antibody (EGFRA) is linked to the PEG via EDC/NHS chemistry on the Fe₃O₄ side. The antibody is used to target A431 human epithelial carcinoma cell line that are known to over-express the epidermal growth factor receptor (EGFR).⁴⁷ The preferred binding between EGFR and EGFRA enables the Au-Fe₃O₄ to be populated on the surface or within the cytoplasm of A431 cells. MRI analyses reveals that A431 cells labeled with 8–20 nm Au-Fe₃O₄ NPs shorten the T₂ relaxation (Figure 8B) with relaxivity $r_2 = 80.4$ mM⁻¹•s⁻¹. A431 cells labeled with 8–20-nm Au-Fe₃O₄ NPs can also be visualized with a scanning confocal microscope at 594 nm, as shown in Figure 8C. The signal detected is from the Au NPs in the Au-Fe₃O₄ structure and reflects the typical morphology of the epithelial cells. The signal is much stronger in the region of cell–cell contact due to the preferred binding between EGFR and EGFRA. This Au NP-based optical probe is very stable and shows no signal loss after three days. The detection limit for the 8–20-nm Au-Fe₃O₄ NPs is 90 pM Au.

Due to their high magnetizations, metallic NPs should have higher T₂ contrasts than the iron oxide NPs for MRI applications. The oleylamine coated 10 nm/2.5 nm bcc-Fe/Fe₃O₄ NPs are made water soluble by the addition of an amphiphilic oleylamine-PEG surfactant.³⁸ Even though these NPs are surrounded by a 15 nm-thick coating, the bcc-Fe/Fe₃O₄ NPs still exhibit a $r_2 = 220$ mM⁻¹•s⁻¹, better than the typical iron oxide NP contrast agent Feridex and comparable with the optimized ferrite NPs with 2 nm-thick coating.¹⁹ The relaxivity is enhanced even greater for the 7 nm FeCo NPs embedded in a single-graphitic-shell and functionalized with phospholipid-PEG.⁴⁶ Their relaxivity reaches 644 mM⁻¹•s⁻¹, nearly 6 times higher than the commercial MRI contrast agent Feridex. FePt NPs with sizes from 4 to 12 nm have been studied as MRI contrast agent.⁴⁸ In this study, cysteamine is used to replace the original surfactants to make FePt NPs water soluble. The MRI contrast is NP size-dependent with larger FePt NPs leading to darker images. These FePt NPs are also promising contrast agent for computed tomography (CT).

4. Magnetic NPs for Therapeutic Applications

The cancer treatment by MFH, based on the sensitivity of cancer cells to heat, has been explored extensively with NPs playing the key role as the local heaters. For 16 nm iron oxide NPs at the superparamagnetism/ferromagnetism transition stage, a hysteresis area $A = 2.3$ mJ•g⁻¹ with $SAR = 1650$ W•g⁻¹ is observed. But for the 4 nm NPs, A is reduced to 0.005 mJ•g⁻¹ with $SAR = 4$ W•g⁻¹.⁴⁹ The advantages of using ferrimagnetic/ferromagnetic NPs with high magnetizations to enhance the NP heating efficiency are further demonstrated by 30 nm Fe₃O₄ NPs,⁵⁰ as well as by 14.8 nm FeCo NPs and 16.3 nm Fe nanocubes.^{51,52} However, these NPs require a strong magnetic field (52 kA•m⁻¹) to achieve magnetic

saturation. This field strength is far above the safety requirement of $16 \text{ kA}\cdot\text{m}^{-1}$ for *in vivo* experiments.¹⁴ The stable weakly ferromagnetic bcc-Fe/Fe₃O₄ NPs have been demonstrated to be efficient for MFH.³⁸ The heating efficiency of these bcc-Fe/Fe₃O₄ is evaluated in a field with $f = 177 \text{ kHz}$ and $\mu_0 H_{\text{max}} = 33 \text{ mT}$, fulfilling the physiological limitations $H_{\text{max}}\cdot f < 5\cdot 10^9 \text{ A}\cdot\text{m}^{-1}\cdot\text{s}^{-1}$, and $f > 50 \text{ kHz}$. Due to their higher magnetization value and small coercive field, the bcc-Fe/Fe₃O₄ NPs have a SAR value of $140 \text{ W}\cdot\text{g}^{-1} \text{ Fe}$. This value is comparable with the ferromagnetic 14 nm FeCo NPs⁵³ and two times higher than the best results reported for 20 nm iron oxide NPs.⁵⁴

5. Conclusions

In this Account, we have reviewed the recent advances in synthesizing monodisperse magnetic NPs of iron oxides, Fe, as well as FePt and FeCo for imaging and therapeutic applications. Due to their ease in fabrication and their approved clinical usage, magnetic Fe₃O₄ NPs with controlled sizes and surface chemistry have been studied extensively for MRI and MFH applications. Porous hollow Fe₃O₄ NPs are expected to have similar magnetic, chemical and biological properties as the solid Fe₃O₄ NPs and have the extra benefits for drug storage and release.²⁹ The Au-Fe₃O₄ NPs combine both magnetically active Fe₃O₄ and optically active Au within one nanostructure and are a promising NP platform for multimodality imaging and therapeutics.

To achieve even higher sensitivity in MRI and efficacy in MFH, magnetic NPs need to have high magnetizations. This has led to the serious search for metallic Fe and FeCo NPs as new probes. However, metallic NPs are normally very reactive and are difficult to be stabilized against fast/deep oxidation in biological solutions. Recent synthetic progress indicates that once coated with a layer of polycrystalline Fe₃O₄ or graphitic shell, these metallic NPs can have much enhanced stability and provide indeed the better contrast for MRI and more effective heating for MFH.

FePt offer a new nano-platform for theranostic applications with FePt showing the composition-dependent magnetism. FePt NPs are chemically more stable than Fe and FeCo NPs and are good contrast agents for not only MRI but also CT. The size and composition dependent magnetism also renders FePt NPs a robust probe with controlled magnetic heating power for MFH applications.

Despite exciting progress, previous studies do not provide satisfactory answers to issues on bio-circulation, bio-distribution and bio-elimination of the magnetic NPs in biological systems. As such issues are related tightly to the interactions between magnetic NPs and biomolecules, understating NP surface coating and NP interactions with various biological components seems to be necessary. Recent advances in NP synthesis make it possible to study biological properties of magnetic NPs and to develop these NPs into practical probes for theranostic applications.

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Biographies

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Figure 1. Schematic illustration of (A) a single domain magnetic NP with its magnetization pointing to one direction, (B) a group of single domain magnetic NPs aligned along a magnetic field direction, (C) the hysteresis loop of a group of ferromagnetic NPs, and (D) the hysteresis loop of a group of superparamagnetic NPs.

Figure 2.

(A) Schematic illustration of the chemical synthesis of Fe₃O₄ NPs, (B) TEM image of a 16 nm Fe₃O₄ NP assembly, and (C) Hysteresis loops of the 16 nm Fe₃O₄ NP assembly measured at 10 K and 300 K. Adapted with permission from references 20 and 21.

Figure 3.

(A) Schematic illustration of the growth of Au-Fe₃O₄ NPs. TEM images of the (B) 6 nm Au NPs and (C) 6–17 nm Au-Fe₃O₄ NPs. Adapted with permission from references 30 and 31.

Figure 4.
(A) Schematic illustration of the formation of FePt NPs from the decomposition of Fe(CO)_5 and reduction of Pt(acac)_2 . TEM images of **(B)** the 6 nm FePt NPs and **(C)** the 9 nm FePt NPs. Adapted with permission from references 39, 40 and 43.

Figure 5.

(A) TEM images of the MnFe₂O₄ NPs (scale bar, 50 nm), (B) T₂-weighted MR images of the corresponding MnFe₂O₄ NPs, (C) color maps of the MR images, and (D) plots of NP size versus relaxivity. Reproduced with permission from reference 19.

Figure 6. Color maps of T₂-weighted MR images of a mouse implanted with the cancer cell line NIH3T6.7 at different time points after injection of the MnFe₂O₄-Herceptin (A–C) and the Fe₃O₄-Herceptin (D–F) conjugates. Reproduced with permission from reference 19.

Figure 7.

(A) Schematic illustration of the coupling of c(RGDyK) peptide to Fe_3O_4 NPs. MRI of the cross section of the U87MG tumors implanted in mice, (B) without NPs, (C) with the injection of 300 μg of c(RGDyK)- Fe_3O_4 NPs. Adapted with permission from reference 26.

Figure 8.

(A) Schematic illustration of the surface functionalization of the Au-Fe₃O₄ NPs, (B) T₂-weighted MRI of (i) 20 nm Fe₃O₄, (ii) 3 nm – 20 nm Au-Fe₃O₄, (iii) 8 nm – 20 nm Au-Fe₃O₄ NPs, and (iv) A431 cells labeled with 8 nm – 20 nm Au-Fe₃O₄ NPs. (C) Reflection image of the 8 nm – 20 nm Au-Fe₃O₄ labeled A431 cells. Adapted with permission from reference 32.