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Chemical Synthesis and Assembly of Uniformly Sized Iron Oxide Nanoparticles for Medical Applications

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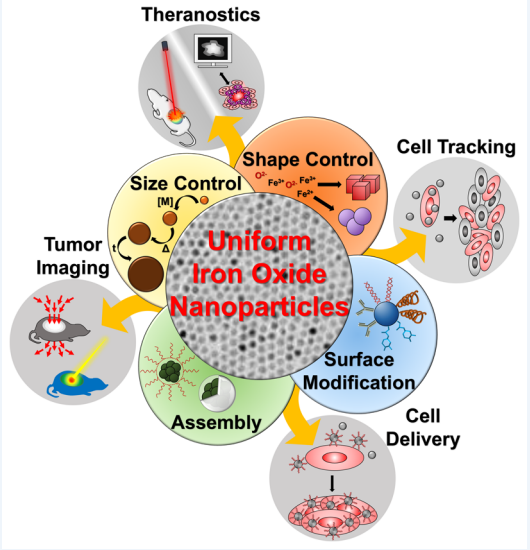
CONSPECTUS: Magnetic iron oxide nanoparticles have been extensively investigated for their various biomedical applications including diagnostic imaging, biological sensing, drug, cell, and gene delivery, and cell tracking. Recent advances in the designed synthesis and assembly of uniformly sized iron oxide nanoparticles have brought innovation in the field of nanomedicine. This Account provides a review on the recent progresses in the controlled synthesis and assembly of uniformly sized iron oxide nanoparticles for medical applications. In particular, it focuses on three topics: stringent control of particle size during synthesis via the "heat-up" process, surface modification for the high stability and biocompatibility of the nanoparticles for diagnostic purposes, and assembly of the nanoparticles within polymers or mesoporous silica matrices for theranostic applications.

Using extremely small 3 nm sized iron oxide nanoparticles (ESION), a new nontoxic T1 MRI contrast agent was realized for high-resolution MRI of blood vessels down to 0.2 mm. Ferrimagnetic iron oxide nanoparticles (FION) that are larger than 20 nm exhibit extremely large magnetization and coercivity values. The cells labeled with FIONs showed very high T2 contrast effect so that even a single cell can be readily imaged.

Designed assembly of iron oxide nanoparticles with mesoporous silica and polymers was conducted to fabricate multifunctional nanoparticles for theranostic applications. Mesoporous silica nanoparticles are excellent scaffolds for iron oxide nanoparticles, providing magnetic resonance and fluorescence imaging modalities as well as the functionality of the drug delivery vehicle. Polymeric ligands could be designed to respond to various biological stimuli such as pH, temperature, and enzymatic activity. For example, we fabricated tumor pH-sensitive magnetic nanogrenades (termed PMNs) composed of self-assembled iron oxide nanoparticles and pH-responsive ligands. They were utilized to visualize small tumors (<3 mm) via pH-responsive T1 MRI and fluorescence imaging. Also, superior photodynamic therapeutic efficacy in highly drug-resistant heterogeneous tumors was observed. We expect that these multifunctional and bioresponsive nanoplatforms based on uniformly sized iron oxide nanoparticles will provide more unique theranostic approaches in clinical uses.



1. INTRODUCTION

Due to unique electrical, magnetic, and optical properties, various kinds of nanoparticles, including semiconductors,[1] magnetic materials,[2,3] and gold,[4] have been extensively investigated for their biomedical applications. In particular, nanoparticles of magnetic iron oxide, including magnetite (Fe_3O_4) and maghemite (γ-Fe_2O_3), have received enormous attention for their various biomedical applications including biosensors, magnetic resonance imaging (MRI) contrast agents, and magnetic fluid hyperthermia[5−11] because they exhibit tunable size-dependent magnetic properties in addition to their benign, nontoxic and biodegradable nature.[3,12]

Magnetite (Fe_3O_4) nanoparticles exhibit unique size-dependent magnetic properties (Figure 1). Bulk magnetite is ferrimagnetic with a Curie temperature of 858 K and multidomain magnetic structure. Magnetite particles become a single domain as the particle size decreases below ∼100 nm, where coercivity is maximized. When the particle size is smaller than ∼20 nm, the magnetization of magnetite nanoparticles is randomized by thermal energy so that they become superparamagnetic. The temperature at which a particle becomes superparamagnetic is called the blocking temperature (T_B) and defined as $T_B = KV/25k_b$ where K is the magnetic anisotropy constant, k_b is the Boltzmann constant, and V is the volume of a nanoparticle. Superparamagnetic iron oxide nanoparticles (SPIO or SPION) have been intensively investigated as T2 MRI contrast enhancement agents. It should be noted that

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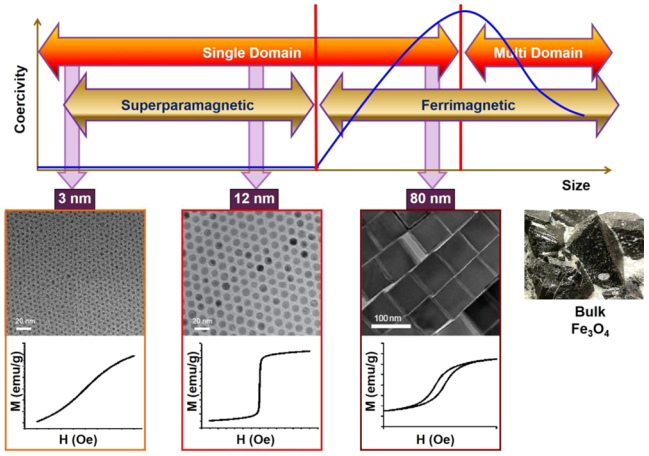

Figure 1. Size-dependent magnetic properties of iron oxide nanoparticles. Inset TEM images and magnetization curves of magnetic nanoparticles were reproduced with permission from refs 15 and 35. Copyright 2011 and 2008 American Chemical Society. And ref 33. Copyright 2004 Nature Publishing Group.

magnetic properties of SPIOs depend on the particle size. For example, uniformly sized SPIOs with the sizes of 4, 6, 9, and 12 nm exhibit saturation magnetization values of 25, 43, 80, and 120 emu g^{-1} Fe, respectively.[13] This size-dependent magnetization of SPIOs, in turn, affects the T2 contrast in MRI. Consequently, synthesis of uniformly sized magnetic nanoparticles is critical for their clinical applications as MRI contrast agents. Actually, it was reported that the poor reproducibility due to the size variation was part of the reason for the withdrawal of dextran-coated SPIOs from American and European markets.[14] Furthermore, when the particle size of iron oxide nanoparticles decreases to <3 nm, most of their magnetic spins are canted and consequently they exhibit nearly paramagnetic behavior.[15]

The particle size also affects cellular uptake, biodistribution, and other pharmacokinetics. Size of iron oxide nanoparticles should be optimized to avoid rapid clearance by the body's immune system, and consequently to be accumulated sufficiently in the target tissue or organ. It was reported that very small-sized nanoparticles (e.g., hydrodynamic diameter of <5.5 nm) are excreted renally,[16] whereas medium-sized nanoparticles enjoy a broad distribution in the bone marrow, heart, kidney and stomach.[11] Meanwhile, large particles with hydrodynamic diameter of >100 nm are readily taken up by phagocytes,[17] and therefore tend to quickly accumulate in fenestrated tissues such as the liver and spleen.[18] Interestingly, nanoparticles of 20−100 nm tend to accumulate in tumors through enhanced permeability and retention (EPR) effect, because cancerous tissues generally have fenestration of blood vessels that are hundreds of nanometers in size.[19,20]

Tremendous advances in the chemical synthetic methods over the past two decades have made it possible to synthesize iron oxide nanoparticles not only with good crystallinity and magnetic properties, but also with uniform sizes of anywhere between a few nanometers and tens of nanometers.[21−23] However, nanoparticles synthesized in organic media are typically capped by hydrophobic ligands, and surface modification is needed to provide the necessary biocompatibility and long-term stability in biological media for various biomedical applications.

Self-assembly of nanoparticles provides a simple and reproducible way to achieve unique and enhanced properties for various applications.[24] For example, assembled magnetic nanoparticles exhibit significantly higher r_2 relaxivity compared to that of well-separated individual nanoparticles.[25,26] Furthermore, designed assembly of iron oxide nanoparticles within polymers and mesoporous silica matrix offers an effective approach to create multifunctional, bioresponsive nanoplatforms for simultaneous diagnostic/imaging and therapeutic (i.e., theranostic) applications.[27−30]

This Account starts with a review of the various methods available for the chemical synthesis of monodisperse iron oxide nanoparticles, with a particular focus on the connection between the size of the particles and their magnetic properties. The role of surface ligands in providing colloidal stability in biological media is then explored along with the effect of ligands on magnetic resonance relaxivity. Chemical approaches for the controlled assembly of iron oxide nanoparticles for targeted imaging and therapy are also discussed, which includes polymeric ligand assisted assembly and the use of mesoporous silica nanoparticles as a template. We put particular attention to the use of iron oxide nanoparticles and their assemblies obtained by these methods in disease diagnostics, biological sensors, gene and drug delivery vehicles, stem cell tracking, and other various biomedical applications. Finally, the remaining challenges for the future clinical translation of uniform iron oxide nanoparticles are closely examined.

2. CHEMICAL SYNTHESIS OF UNIFORM IRON OXIDE NANOPARTICLES

Although numerous chemical methods have been developed for the synthesis of iron oxide nanoparticles,[2,3] we herein focus on a representative one called "heat-up" method, which was pioneered by the Hyeon group for the scalable synthesis of highly uniform iron oxide nanoparticles suitable for medical applications. This heat-up method involves slow heating of a reaction mixture composed of precursors, surfactants, and high-boiling solvent from room temperature to a high temperature (Figure 2a). In the original report on the heat-up method in 2001, iron pentacarbonyl precursor is mixed with oleic acid and octyl ether at 100 °C, and then slowly heated to the boiling temperature of ∼320 °C.[31] Despite its simplicity, this method can produce highly monodisperse iron oxide nanoparticles without going through a separate size sorting process, which is attributed to an intermediate step between the decomposition of the in situ generated iron−oleate complex and the formation of the final nanoparticles. Consequently, these iron-oxo cluster intermediate species, rather than the iron−oleate complex itself, seem to act as monomers for the generation of iron oxide nanocrystals. The overall formation mechanism of the heat-up process consists of the following steps: the generation and accumulation of monomers, burst nucleation, and the diffusion-controlled growth process, which are in accordance with the LaMer model for monodisperse microspheres.[32] Since thermally stable intermediate species play an important role in the formation of uniform-sized nanoparticles, it allows a wide range of precursors, including cheap and environmentally benign metal salts, to be used. This eventually led to the large-scale synthesis of uniform iron oxide nanocrystals via an improved heat-up method using iron−oleate complex precursor, which was easily obtained from a simple reaction between iron chloride and sodium oleate.[33]

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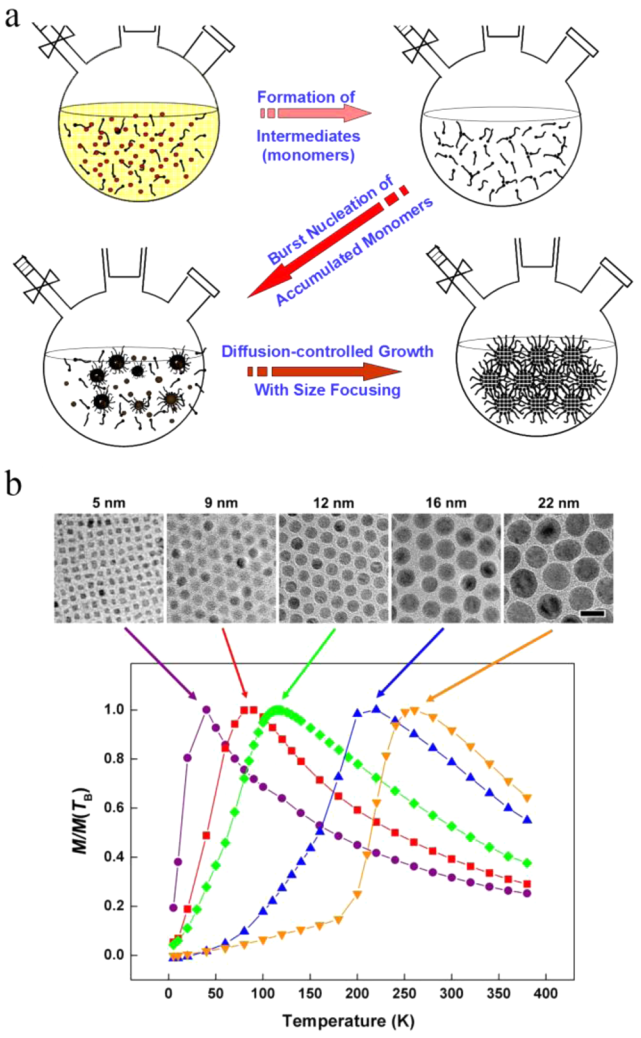

Figure 2. (a) Schematic illustration of the "heat-up" method for the synthesis of uniformly sized iron oxide nanoparticles. (b) TEM images of iron oxide nanoparticles synthesized by the "heat-up" method and a plot of the zero-field-cooling magnetization (M) of these iron oxide nanocrystals. Reproduced with permission from ref 33. Copyright 2004 Nature Publishing Group.

As shown by the magnetic property data in Figure 2b, iron oxide nanocrystals produced by the heat-up method show remarkable size uniformity with σ of <5%. Moreover, the particle diameter can be readily controlled by using solvents with different boiling points. Iron-oleate complex could be synthesized by other routes including the reaction of either FeO(OH) with oleic acid[34] or ferric and ferrous chlorides with oleic acid.[22] More precise size control of the nanoparticles in one nanometer scale was also achieved by the seed-mediated growth process using as-synthesized monodisperse iron nanoparticles as seeds.[23] The heat-up method was later used to synthesize uniform cube-shaped ferrimagnetic iron oxide nanoparticles with controlled sizes ranging from 20 to 160 nm by using a reaction mixture composed of iron(III) acetylacetonate, oleic acid, and benzyl ether.[35] Uniform and extremely small (<4 nm) iron oxide nanoparticles were also synthesized by the thermal decomposition of iron−oleate complex in the presence of oleyl alcohol.[15] In this synthesis, oleyl alcohol acts as a mild reductant and helps to lower the reaction temperature, making it possible to control the particle size from 1.5 to 3.7 nm by simply changing the aging temperature or the ratio of oleyl alcohol to oleic acid. More recently, matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (MS) was employed to characterize the size distribution of these ultrasmall-sized iron oxide nanoparticles with very high accuracy and time efficiency compared to the conventional characterization methods such as transmission electron microscopy (TEM).[36]

3. SURFACE MODIFICATION

For various biomedical applications, uniform iron oxide nanoparticles synthesized by the heat-up method in hydrophobic solvents need surface modification processes to achieve biocompatibility and long-term stability in biological media. Two most popular processes, ligand exchange and encapsulation with amphiphilic polymers, have been employed to produce water-dispersible and biocompatible iron oxide nanoparticles. Furthermore, multiple interaction ligands have been synthesized by combining advantageous features of the ligand exchange and the encapsulation processes to get highly robust water-dispersible iron oxide nanoparticles. Herein, we will summarize our group's efforts on the surface modification of uniform iron oxide nanoparticles synthesized by the heat-up method and their applications to MRI contrast agents.

3.1. Ligand Exchange

The ligand exchange of hydrophobic capping ligand with new hydrophilic ligand is the most popular approach to transform hydrophobic nanoparticles synthesized in organic solvents into water-dispersible and biocompatible nanoparticles. In this approach, the binding ability of the hydrophilic ligand to the nanoparticle surface is crucial to ensure a stable dispersion. Hydrophilic ligands with the anchoring groups such as dopamine,[37] carboxylic acids,[38] phosphines,[39] and amines[40] generally have a good affinity for iron oxide surface. However, the excess amount of the ligands is often needed for the ligand exchange reaction.

PEG-derived phosphine oxide (PO-PEG) ligand has been demonstrated to displace oleic acid ligand in the iron oxide nanoparticles synthesized by the heat-up method (Figure 3a) to produce water-dispersible iron oxide nanoparticles.[41] Moreover, a simple reaction with bifunctional reagents such as 1,2-ethylenediamine can endow these PO-PEG ligands with active functional groups that allow them to conjugate with imaging dyes or targeting agents. Iron oxide nanoparticles coated with PO-PEG ligand are also suitable for cellular labeling since their spin−spin relaxation times (T_2) are significantly shortened.[41] Extremely small-sized iron oxide nanoparticles (ESION) can also be transferred to aqueous media through ligand-exchange with PO-PEG ligands.[15] The resulting water-dispersible ESIONs with a diameter of 3 nm exhibit a high r_1 relaxivity of 4.78 mM^{-1} s^{-1} and a low r_2/r_1 ratio of 6.12 (Figure 3b). In addition to the efficient T1 MR contrast agent, PO-PEG coated ESIONs can also be used as high-resolution blood pool MR imaging agent with their circulation time longer than that of the gadolinium complex-based contrast agents that are currently used in the clinic (Figure 3c).

Catechol-functionalized polypeptides have been developed for the ligand exchange of hydrophobic iron oxide nanoparticles and subsequent immobilization of fluorescence dyes.[42] The resulting multimodal transfection agents (MTAs) with a hydrodynamic diameter of ∼40 nm exhibit greatly enhanced colloidal stability in aqueous media. More importantly, the

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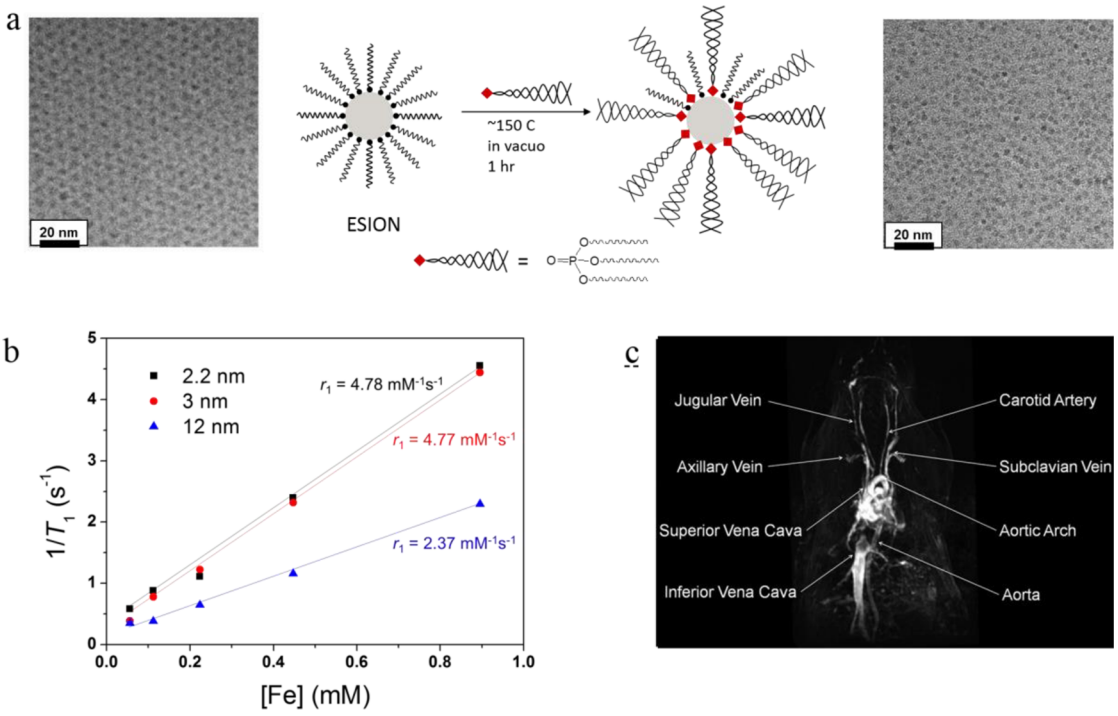

Figure 3. (a) Schematic representation of ligand exchange strategy for extremely small iron oxide nanoparticles (ESIONs) using PO-PEG ligands and a TEM image of ESIONs before and after the ligand exchange. (b) Plot of $1/T_1$ over Fe concentration of iron oxide nanoparticles with diameters of 2.2 nm (■), 3 nm (red ●), and 12 nm (blue ▲). The slope indicates the specific relaxivity (r_1). (c) High resolution blood pool MR image following intravenous injection of ESIONs. Reproduced with permission from ref 15. Copyright 2011 American Chemical Society.

nanoparticles are capable of binding DNA molecules to stem cells for gene transfection, and exhibit an enhanced efficiency in human mesenchymal stem cells (hMSCs) compared with commercial transfection agents such as Lipofectamine. This is attributed to the high r_2 relaxivity of MTAs (~169.09 mM^{-1} s^{-1}) along with the immobilized fluorescence dyes, allowing MTA-treated hMSCs to be visualized in vivo for 14 days after transplantation through both MR and optical imaging.

3.2. Ligand Encapsulation

Encapsulation of nanoparticles within self-assembled amphiphilic polymers is another popular approach to get biocompatible nanoparticles for various medical applications. Among various kinds of polymers, United States Food and Drug Administration (FDA)-approved PEG-terminated phospholipid is widely used, where the phospholipid-based ligand is bound to nanocrystal surface and subsequently forms micelles via hydrophobic van der Waals interaction, while the outer PEG chain provides colloidal stability in aqueous media (Figure 4a,b).[43] Because the molecular weight of the phospholipid-PEG ligands determines the shell thickness of the resulting nanoparticles, it plays an important role in determining the relaxivity of the iron oxide nanoparticles. For example, the relaxivity of iron oxide nanoparticles with core diameter of 14 nm and encapsulated within 1000 Da PEG-terminated phospholipid is 2.54 times higher than that encapsulated with 5000 Da PEG-phospholipid.[44] This encapsulation method can also be used to produce water-dispersible ferrimagnetic iron oxide nanocubes (FIONs),[45,46] which are otherwise very unstable in aqueous media and easily form aggregates due to their high magnetic attractive force. Although 60 nm core sized FIONs encapsulated within 2000 Da phospholipid-PEG ligand coated FIONs in diameter are not that stable in aqueous media, they can still be used for MRI-based in vivo cell tracking as their high relaxivity (r_2) of 324 mM^{-1} s^{-1} provides single-cell level sensitivity.[45] These FIONs have been used to image intrahepatically transplanted pancreatic islets in a swine model by a 1.5 T clinical MRI scanner, which has significant implication for the treatment of type 1 diabetes mellitus. Furthermore, the theoretically predicted maximum r_2 relaxivity could be achieved by optimizing the size of FIONs (Figure 4c). FIONs with core dimension of 22 nm and encapsulated with 2000 Da phospholipid-PEG exhibit an extremely high r_2 relaxivity of 761 mM^{-1} s^{-1} and high colloidal stability in aqueous media.[46] Accumulation of FIONs at tumor sites after intravenous injection allows the high resolution in vivo MR imaging of tumors using a clinical 3 T MR scanner (Figure 4d).

In addition to PEG-based ligands, natural polymers such as polysaccharides and polypeptides have been used as stabilizers for iron oxide nanoparticles. Among these kinds of ligands, l-3,4-dihydroxyphenylalanine (DOPA)-conjugated chitosan oligosaccharide is of particular interest as this has a high affinity for FIONs.[47] Indeed, DOPA-chitosan coated 30 nm sized FIONs have shown to be not only stable in water, but also to exhibit a much better hyperthermia efficiency than commercial SPIOs due to their higher magnetization and low coercivity.

3.3. Multiple Interaction Ligands

Because both the ligand exchange and the encapsulation processes are used to change hydrophobic nanoparticles into water-dispersible and biocompatible nanoparticles, synergistic combination of these two characteristics within one ligand system will produce highly robust and water-dispersible nanoparticles for various biomedical applications. Using a naturally occurring mussel-adhesive protein as basis, poly(l-3,4-dihydroxyphenylalanine) (polyDOPA)-based multiple interaction ligands (MILs) have been developed to produce versatile and ultrastable nanoparticles of various materials including iron

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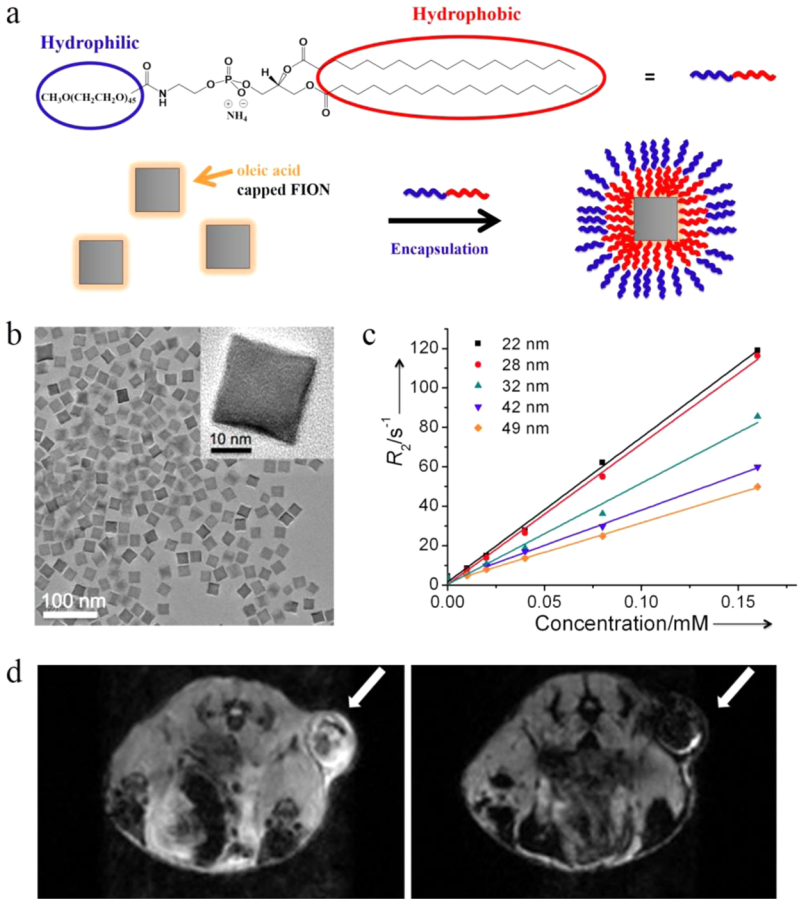

Figure 4. (a) Schematic representation of ligand encapsulation strategy for FIONs using lipid-PEG ligands. (b) TEM image of 22 nm FIONs dispersed in water (inset: HRTEM image). (c) Plots of R2 values of FIONs of different core sizes. FIONs with core dimension of 22 nm and encapsulated with 2000 Da phospholipid-PEG exhibit an extremely high r_2 relaxivity of 761 mM^{-1} s^{-1}. (d) In vivo MR images of a tumor site before (left) and 1 h after (right) intravenous injection of FIONs (arrows indicate the tumor site) showing the significant attenuation in the MR signal. Reproduced with permission from ref 46. Copyright 2012 American Chemical Society.

oxide.[48] These MILs contain catechol and amine end groups for direct binding with iron oxide nanoparticles, while amphiphilic branched block copolymer moiety forms micelles that encapsulate the nanoparticles. In addition, the positive charge of the ligand allows for electrostatic interaction with most negatively charged nanoparticle surfaces (Figure 5a). The resulting MIL-coated iron oxide nanoparticles exhibit an extremely high stability in various harsh aqueous environments, including highly acidic and basic media, highly concentrated NaCl solution and even boiling water. Due to this excellent stability, MIL-coated nanoparticles have a relatively long blood half-life in mice compared with the previously reported iron oxide-based nanoparticles.[49,50] As a result, they can be accumulated in the lymph nodes of a nude mouse 24 h after intravenous injection, which was clearly detected by T2 weighted MRI (Figure 5b,c).

4. ASSEMBLY OF UNIFORM IRON OXIDE NANOPARTICLES

Designed assembly of nanoparticles provides a simple approach to have unique properties that are not available from separated nanoparticles and bulk materials.[24] For example, assembled SPIOs exhibit significantly higher r_2 relaxivity than well-dispersed individual nanoparticles.[25,26] Furthermore, the self-assembly of iron oxide nanoparticles within polymers and mesoporous silica matrices will develop novel multifunctional bioresponsive nanoplatforms for theranostic applications.[27−30]

4.1. Assembly of Iron Oxide Nanoparticles with Mesoporous Silica Particles

Due to their unique characteristics including high surface areas, tunable nanometer-sized pores, and well-defined surface properties, mesoporous silica nanoparticles (MSNs) have been extensively used as multifunctional platform materials for incorporating various functional nanostructured materials including iron oxide nanoparticles.[28−30] Among several different kinds of immobilization methods, two approaches, the encapsulation of iron oxide nanoparticles in mesoporous silica shell and the assembly of the nanoparticles on the silica surface, will be highlighted.

Uniform-sized iron oxide nanoparticles were immobilized on the surface of mesoporous dye-doped silica nanoparticles, and the resulting nanocomposite particles with diameter of ∼100 nm (termed Fe_3O_4-MSN) were applied as simultaneous MRI, fluorescence imaging and as a drug delivery vehicle.[51] For the assembly process, bromo-functionalized Fe_3O_4 nanoparticles were reacted with amine-terminated silica particles to produce the nanocomposite particles. The r_2 relaxivity is 2.8 times greater than that of dispersed Fe_3O_4 nanoparticles of the same diameter due to the clustering effect of magnetic nanoparticles. In addition to being useful for MRI, therapeutic agents can also

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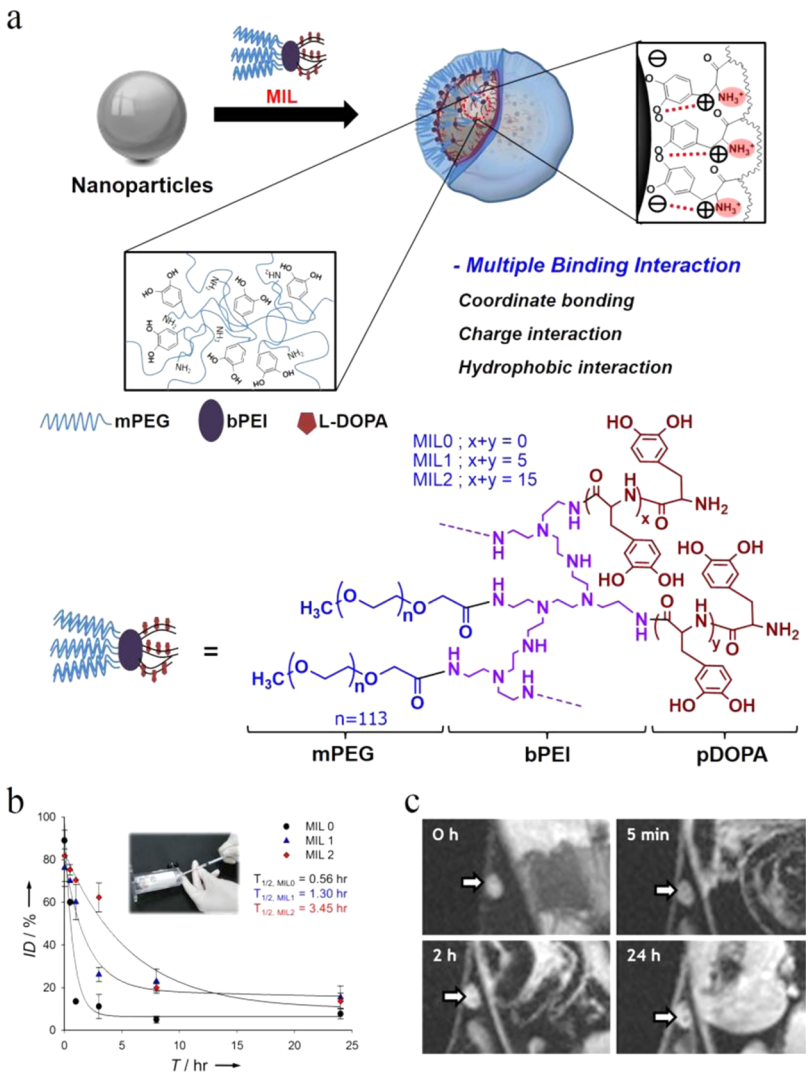

Figure 5. Stabilization of nanoparticles through multiple-interaction ligands. (a) Formation of water-dispersible nanoparticles by multiple-interaction ligand (MIL) stabilization. (b) In vivo stability tests: blood circulation data (plasma iron concentration versus time relationships) of MIL0, MIL1, and MIL2-functionalized Fe_3O_4 nanoparticles (inset). (c) Time-dependent T_2 weighted MR images of the lymph node (arrow) in a nude mouse before and after intravenous administration of MIL2-functionalized Fe_3O_4 nanoparticles. Reproduced with permission from ref 48. Copyright 2011 Wiley-VCH.

be loaded into the pores of the mesoporous silica to provide a multifunctional nanoplatform capable of simultaneous imaging and therapeutic modalities. The signal drop in T_2-weighted MR image obtained 3 h after the intravenous injection of Fe_3O_4-MSN has confirmed that it can accumulate in a tumor, while the immobilized fluorescent doxorubicin (DOX) allows the tumor sites to be directly visualized by fluorescence microscopy. Furthermore, apoptotic morphology was clearly visualized in the tumor sites, demonstrating that DOX was effectively delivered to exhibit good anticancer activity.

Iron oxide nanoparticles can be also encapsulated within mesoporous silica matrix by the synthesis of mesoporous silica in the presence of the core nanoparticles.[52] Uniform-sized core/shell nanoparticles composed of monodisperse iron oxide nanoparticle core and uniform mesoporous silica shell were fabricated by the controlled silica sol–gel reaction in the presence of iron oxide nanoparticles synthesized by the heat-up method. After PEGylation, the resulting multifunctional nanoparticles were used as simultaneous MRI, fluorescence imaging agents, and drug delivery vehicle.[52]

4.2. Assembly of Iron Oxide Nanoparticles via Enzymatic Reaction

Selective assembly of iron oxide nanoparticles on the surface of cancer cells via enzymatic reactions can be used to sort and target cancer cells. For example, iron oxide nanoparticles capped with D-tyrosine phosphate can be catalytically dephosphorylated by ectophosphatases of cancer cells to form tyrosine-coated nanoparticles, which are selectively assembled on the surface of cancer cells. Consequently, the cancer cells immobilized with iron oxide nanoparticles can be easily separated from the mixture of cancer and stromal cells by using a small magnet. Furthermore, the growth of cancer cells can be inhibited by the surface-bound iron oxide nanoparticles.[53]

4.3. Immobilization of Iron Oxide Nanoparticles within Polymers

Iron oxide nanoparticles can be immobilized in polymer matrices to fabricate multifunctional nanocomposite particles for targeted theranostic applications. For example, unifrom iron oxide nanoparticles could be immobilized within poly(D,L-

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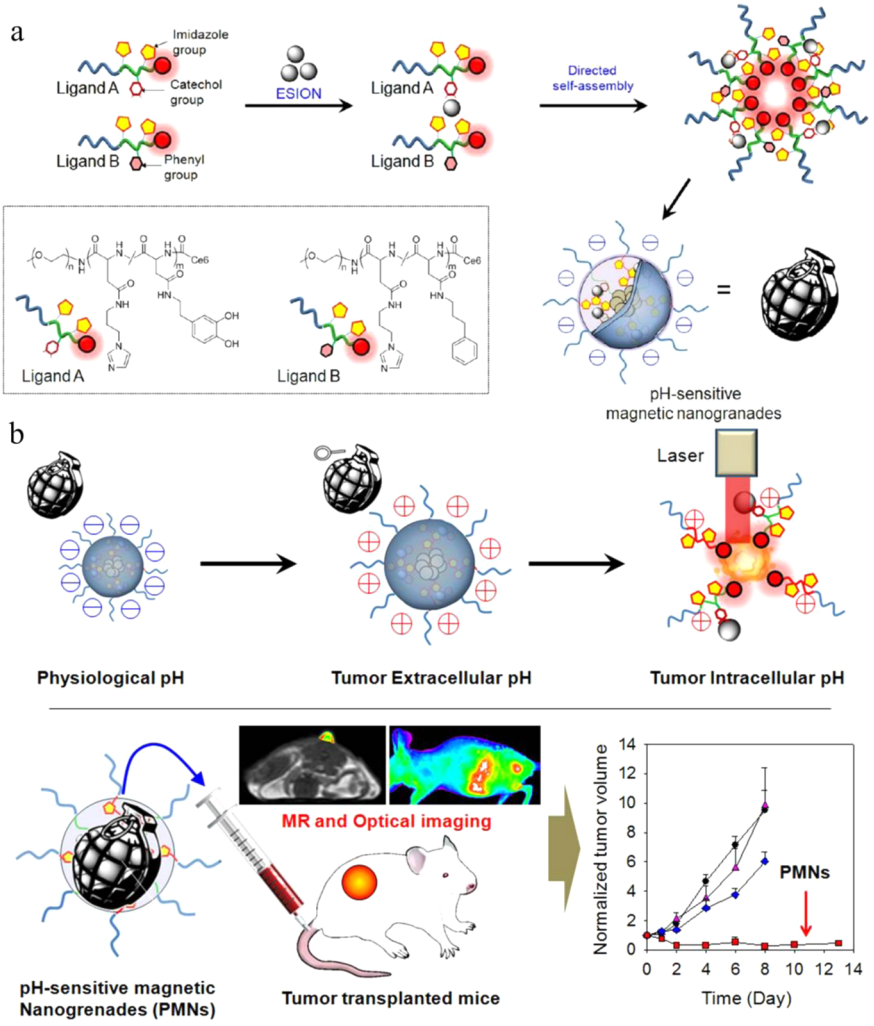

Figure 6. Design and mechanism of pH-sensitive magnetic nanogrenades (PMNs) for tumor-pH activation: (a) Schematic representation of the pH-responsive ligand-assisted self-assembly of extremely small iron oxide nanoparticles (ESIONs). (b) Schematic representation of a tumor pH-recognizable treatment strategy using PMNs and subsequent in vivo tumor imaging and therapy results. Reproduced with permission from ref 57. Copyright 2014 American Chemical Society.

lactic-*co*-glycolic acid) (PLGA) by using a microemulsion method.[54] After the conjugation of cancer-targeting folate ligand, the polymer nanoparticles were effectively accumulated in KB cancer cells that overexpress folate receptors, and the subsequent application of an external magnetic force enhances the MRI signal and cytotoxicity.

Polymeric matrices can be designed to endow the assembled iron oxide nanostructures with stimuli-responsive characteristics for smart theranostic applications.[55,56] We fabricated tumor pH-sensitive magnetic nanogrenades (termed PMNs) composed of self-assembled iron oxide nanoparticles and pH-responsive polymers for ultrasensitive bimodal imaging and treatment of resistant heterogeneous tumors in vivo (Figure 6).[57] PMNs can selectively target tumors via surface-charge reversal triggered by the acidic tumor microenvironment, and are later disassembled into a highly active state in acidic subcellular compartments that "turns on" MRI signal, fluorescence and photodynamic activity. We could image very small tumors of <3 mm in mice via simultaneous pH-sensitive T1 MRI and fluorescence imaging. Furthermore, pH-triggered photodynamic therapeutic activity could selectively kill cancer cells, in particular exhibiting excellent therapeutic efficacy in highly heterogeneous drug-resistant tumors. This pH-responsive polymer was used to immobilize potent cancer drug, triptolide, which has not only poor solubility in body fluid but also excessively high toxicity.[58] After folate conjugation, the resulting pH-sensitive nanoformulated triptolide could facilitate efficient accumulation and subsequent treatment of hepatocellular carcinoma, a cancer with one of the worst prognoses for survival.

5. CONCLUSIONS AND PERSPECTIVES

This Account summarizes recent advances on the synthesis of uniform-sized iron oxide nanoparticles by the heat-up method, their surface modification for MRI contrast agent applications, and designed assembly for multifunctional theranostic applications. Size-dependent magnetic characteristics of uniform-sized iron oxide nanoparticles are able to develop various kinds of MRI contrast agents. For example, using nearly paramagnetic property of 3 nm sized iron oxide nanoparticles, a new nontoxic T1 MRI contrast agent was realized for high resolution MRI of blood vessels down to 0.2 mm. On the other hand, 50 nm ferrimagnetic iron oxide nanocubes were employed as MRI contrast agents for imaging single cells and tracking transplanted pancreas islets to treat type-1 diabetes

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mellitus. Iron oxide nanoparticles are immobilized in mesoporous silica particles or polymers for simultaneous MRI, fluorescence imaging, and drug delivery/therapy. For example, tumor pH-sensitive magnetic nanogrenades composed of self-assembled iron oxide nanoparticles and pH-responsive polymer were fabricated not only for imaging small tumors of <3 mm via pH-responsive T1 MRI and fluorescence imaging but also for superior photodynamic therapeutic efficacy in highly drug-resistant heterogeneous tumors.

Iron oxide based nanomaterials have great potential for clinical applications due to their biocompatibility and well-characterized pharmacokinetics. In particular, the large-scale synthesis of uniform-sized iron oxide nanoparticles, their surface modification, and their assembly have come a long way in overcoming the difficulties that have so far prevented their clinical applications. Ongoing and future efforts therefore need to focus on providing a better understanding of the biological interactions between engineered iron oxide nanoparticles and clinical-relevant disease models in large animals and eventually humans, and to investigate other related key issues, such as long-term biocompatibility, biodegradability, and pharmacokinectics. To achieve these ambitious goals of clinical translation, close multidisciplinary and interdisciplinary collaboration among chemistry, biology, pharmacy, and preclinical and clinical medicine should be conducted.

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Notes

The authors declare no competing financial interest.

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Nohyun Lee received his Ph.D. (2011) in Chemical and Biological Engineering from Seoul National University with the supervision of Prof. Taeghwan Hyeon. During his doctorate period, he has been working on the development of new MRI contrast agents based on iron oxide nanoparticles. He is currently an assistant professor of School of Advanced Materials Engineering at Kookmin University

Taeghwan Hyeon received his B.S. (1987) and M.S. (1989) in Chemistry from Seoul National University. He obtained his Ph.D. (1996) in Chemistry from University of Illinois at Urbana–Champaign. Since he joined the faculty of the School of Chemical and Biological Engineering of SNU in September 1997, he has focused on the synthesis and applications of uniform-sized nanocrystals and nanoporous materials. He is a Director of Center for Nanoparticle Research in the Institute for Basic Science (IBS). He is currently serving as an Associate Editor of *J. Am. Chem. Soc.*, and an editorial advisory board member of *Adv. Mater.*, *Nano Today*, *Nanoscale*, and *Small*.

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