ACCOUNTS
of chemical research

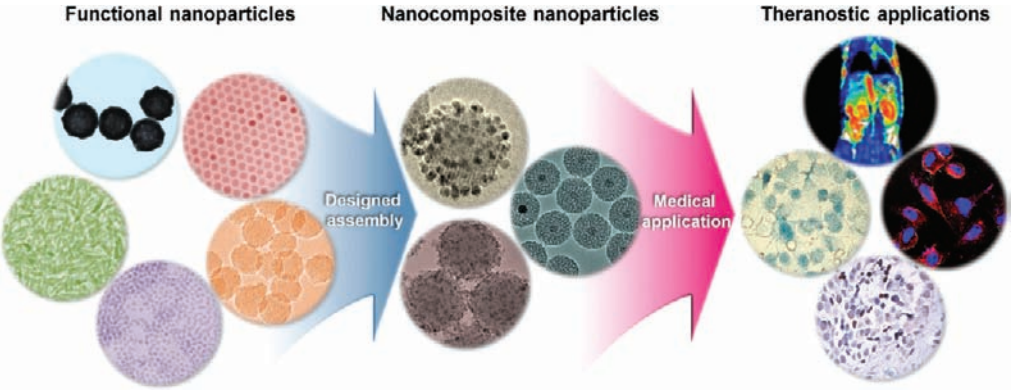
Multifunctional Mesoporous Silica Nanocomposite Nanoparticles for Theranostic Applications

JI EUN LEE, NOHYUN LEE, TAEHO KIM, JAEYUN KIM, AND TAEGHWAN HYEON*

World Class University Program of Chemical Convergence for Energy & Environment, and School of Chemical and Biological Engineering, Seoul National University, Seoul 151-744, Korea

RECEIVED ON FEBRUARY 1, 2011

CONSPECTUS



Clever combinations of different types of functional nanostructured materials will enable the development of multifunctional nanomedical platforms for multimodal imaging or simultaneous diagnosis and therapy. Mesoporous silica nanoparticles (MSNs) possess unique structural features such as their large surface areas, tunable nanometer-scale pore sizes, and well-defined surface properties. Therefore, they are ideal platforms for constructing multifunctional materials that incorporate a variety of functional nanostructured materials.

In this Account, we discuss recent progress by our group and other researchers in the design and fabrication of multifunctional nanocomposite nanoparticles based on mesoporous silica nanostructures for applications to simultaneous diagnosis and therapy. Versatile mesoporous silica-based nanocomposite nanoparticles were fabricated using various methods. Here, we highlight two synthetic approaches: the encapsulation of functional nanoparticles within a mesoporous silica shell and the assembly of nanoparticles on the surface of silica nanostructures. Various nanoparticles were encapsulated in MSNs using surfactants as both phase transfer agents and pore-generating templates. Using MSNs as a scaffold, functional components such as magnetic nanoparticles and fluorescent dyes have been integrated within these systems to generate multifunctional nanocomposite systems that maintain their individual functional characteristics. For example, uniform mesoporous dye-doped silica nanoparticles immobilized with multiple magnetite nanocrystals on their surfaces have been fabricated for their use as a vehicle capable of simultaneous magnetic resonance (MR) and fluorescence imaging and drug delivery. The resulting nanoparticle-incorporated MSNs were then tested in mice with tumors. These in vivo experiments revealed that these multifunctional nanocomposite nanoparticles were delivered to the tumor sites via passive targeting. These nanocomposite nanoparticles served as successful multimodal imaging probes and also delivered anticancer drugs to the tumor site. With innumerable combinations of imaging modalities and drug delivery available within these vehicles, multifunctional nanocomposite nanoparticles provide new opportunities for dinical diagnostics and therapeutics.

1. Introduction

Recently, various nanostructured materials have been investigated for their potential applications in biomedical imaging, diagnostics, and therapy. The novel size-dependent physical properties of nanostructured materials along with the nanometer-scale dimension of many important biological systems make them highly attractive for many biomedical applications.[1−4] For example, semiconductor nanoparticles

Published on the Web 08/17/2011 www.pubs.acs.org/accounts
10.1021/ar2000259

have been used for targeted fluorescence imaging of tumor as well as long-term real-time imaging of molecular events in cells.[5,6] Gold nanoparticles conjugated with oligonucleotides are capable of sensing complementary DNA strands based on color changes resulting from a shift in surface plasmon resonance wavelength.[7] Magnetic nanoparticles have been used in various biomedical applications such as contrast agents for magnetic resonance imaging (MRI),[8−11] and bioseparation.[12] Various gold nanostructures including nanoshells and nanorods have been intensively studied and developed for noninvasive photothermal therapy.[13,14]

In addition to these unique chemical and physical properties of the nanoparticles, the nanosize itself makes them ideal platforms for the biomedical applications. Due to their large surface areas, nanoparticles can be readily conjugated with molecular moieties capable of recognizing various complementary biomolecules including DNA strands and antigens with high sensitivity and selectivity, which is advantageous in targeted imaging, diagnosis, and delivery.[2,3] This nanometer dimension becomes even more important when the nanoparticles are systemically administered into living organisms because nanoparticles can readily internalized into cells, followed by contacting with subcellular organelles[4] and release of cargos.[15] The circulation time and interactions of nanoparticles with various cells are highly dependent on the size and surface characteristics of the nanoparticles. In the bloodstream, micrometer-sized particles and small molecules exhibit relatively short circulation times because they are rapidly removed by active phagocytosis of the reticuloendothelial system (RES) and renal clearance, respectively.[4,16] In contrast, well-stabilized nanoparticles with optimal size and appropriate antifouling surface can remain in blood vessels long enough to accumulate at the tumor sites via enhanced permeation and retention (EPR) effect, which maximizes their performance in targeted imaging or therapy.[17]

Clever combinations of different kinds of functional nanostructured materials will enable the development of multifunctional nanomedical platforms for multimodal imaging or simultaneous diagnosis and therapy (referred as theranostics).[18−20] Theranostic agents based on nanoparticles will enable monitoring of circulation and biodistribution of drug delivery carrier. In addition, these integrated systems will allow delivery of therapeutic agents to target tissues selectively via passive targeting derived from their nanosize, and simultaneous real-time noninvasive monitoring of biological responses to the therapy, which can provide important feedback in the treatment of disease.

Among various integrated nanocomposite systems, mesoporous silica-based nanostructured materials have attracted great interest since they exhibit low cytotoxicity and excellent chemical stability and their surface can be easily modified.[21,22] Moreover, the large surface area and pore volume of mesoporous silica ensure facile adsorption as well as high loading of various therapeutic materials.[23,24] In terms of biocompatibility, silica is accepted as "Generally Recognized As Safe" (GRAS) by the United States Food and Drug Administration (FDA). Very recently, dye-doped silica nanoparticles, called Cornell dots (C dots), have received approval from the FDA for the first Investigational New Drug (IND) application for targeted molecular imaging of cancer.[25] Furthermore, it was reported that mesoporous silica nanoparticles (MSNs) exhibit lower hemolytic activity compared to their nonporous, similarly sized counterparts,[26,27] suggesting that MSNs are suitable for systemic delivery through the bloodstream. Several in vivo biodistribution studies of MSNs have been reported recently.[28,29] Using noninvasive optical imaging techniques, Lee et al. reported that fluorescently labeled MSNs tended to accumulate rapidly in the liver when they were administrated via intravenous injection into nude mouse.[28] Lu et al. investigated biocompatibility and biodistribution of MSNs in a human cancer xenograft mouse[29] and showed that MSN is biocompatible at the effective dosages and preferentially accumulated in the tumor. Moreover, treatment with drug-loaded MSNs resulted in suppression of tumor growth in mice, demonstrating the drug-delivery capability of MSNs.

More recently, the pore surface and opening of MSNs have been functionalized with stimuli-responsive groups, inorganic nanoparticles, supramolecules, and proteins that can work as caps and gatekeepers.[23,24,30−38] "On-demand" controlled release of encapsulated drugs can be triggered in response to internal or external stimuli such as pH, temperature, redox potential, light, and enzyme reactions.[30−38] In addition, MSNs modified with fluorescent dye and paramagnetic metal complex were used as fluorescent and magnetic imaging probes.[28,39] There are several excellent review articles on the biomedical applications of MSNs.[23,24,40,41]

In this Account, we discuss our group's recent progress made in the designed fabrication of mesoporous silica-based nanocomposite nanoparticles for multifunctional theranostic applications. Among several different kinds of fabrication methods for nanocomposite nanoparticles, two approaches, including encapsulation of functional nanoparticles in mesoporous silica shell and assembly of nanoparticles on the surface of silica nanostructures, will be highlighted.

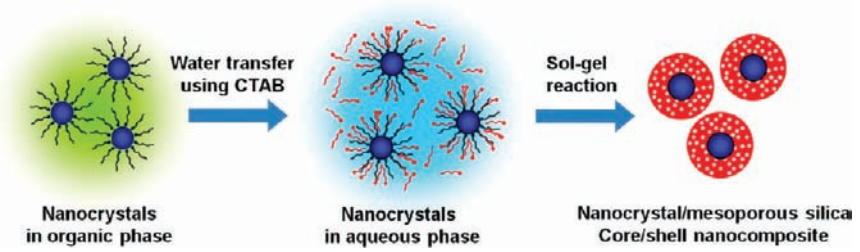


FIGURE 1. Synthetic procedure for encapsulation of hydrophobic nanoparticles with mesoporous silica shell. Reproduced from ref 45. Copyright 2008 Wiley-VCH.

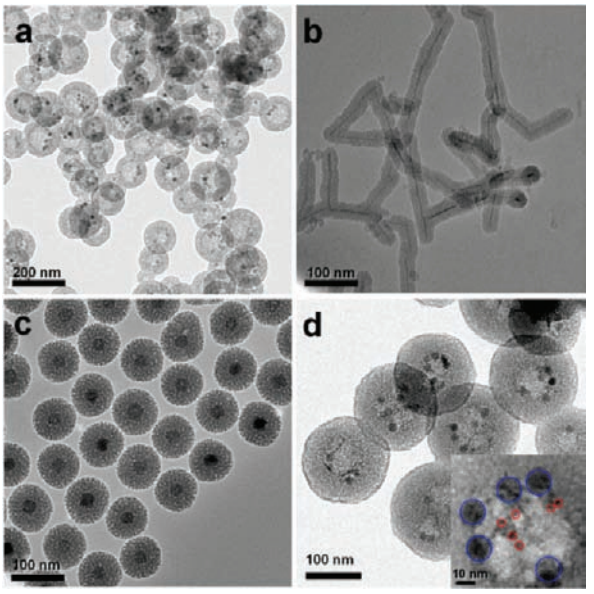


FIGURE 2. Transmission electron microscopy (TEM) images of various nanocrystal-embedded mesoporous silica nanostructures. (a) Iron oxide nanoparticle-embedded MSNs. (b) One-dimensional α-FeOOH nanotubes sheathed with mesoporous silica shell. (c) Manganese oxide nanoparticle-embedded MSNs. (d) Iron oxide nanoparticle and quantum dot coembedded MSNs. Inset: magnified image. Quantum dots (red circles) and iron oxide nanoparticles (blue circles). Reproduced from refs 44 and 45. Copyright 2006 American Chemical Society. Copyright 2008 Wiley-VCH.

2. Multifunctional Mesoporous Silica Nanocomposite Nanoparticles

2.1. Nanoparticles Encapsulated within Silica Shell. Encapsulation with silica matrix is one of the most widely used methods for surface modification of inorganic nanoparticles,[42] because the unique properties of the nanoparticles can be preserved by silica shells and various high quality nanoparticles synthesized in organic media[43] can be readily transferred to aqueous media. Controlled sol–gel reactions generate amorphous silica shell around the nanoparticles. The formation of mesoporous silica instead of a dense silica shell can impart additional drug delivery functionality onto silica-based nanocomposite nanoparticles.

In 2006, the Hyeon group reported a simple and general method for encapsulation of inorganic nanoparticles in mesoporous silica shell (Figure 1).[44] Cetyltrimethylammonium bromide (CTAB) could be used not only as a phase transfer agent but also as a template for mesopore generation for uniform nanoparticles encapsulated in mesoporous silica shell. In this method, presynthesized uniform iron oxide nanoparticles stabilized with oleic acid were transferred to aqueous media by capping with CTAB. Subsequent silica sol–gel reaction followed by the removal of surfactants resulted in the production of mesoporous silica spheres embedded with iron oxide nanoparticles (Figure 2a). This synthetic method could be generally applied to other hydrophobic nanoparticles of various compositions and shapes for their coating with mesoporous silica shells. For example, as shown in Figure 2b and c, one-dimensional α-FeOOH nanotubes and spherical MnO nanoparticles could be also embedded in mesoporous silica shell. Moreover, different kinds of nanocrystals, such as iron oxide nanoparticles and quantum dots, could be simultaneously immobilized in a single mesoporous silica sphere (Figure 2d). Since the encapsulated nanoparticles retained their original physical properties, the resulting nanocomposite nanoparticles showed both magnetic and fluorescent properties. This simple and highly reproducible synthetic process can serve as a standard protocol for the fabrication of uniform-nanoparticle/mesoporous-silica core/shell nanostructures for multifunctional theranostic applications.

Using this synthetic method, uniform iron-oxide-nanoparticle/mesoporous-silica core/shell nanocomposite nanoparticles (Fe_3O_4@mSiO_2) were synthesized for imaging and therapy.[45] Particle size strongly affects the efficiency of in vivo delivery and cellular uptake.[16,46] For effective systemic delivery, it is desirable to keep the size of therapeutic nanocomposite particles smaller than 100 nm because of their high colloidal stability in a physiological environment and long blood circulation time. By optimizing the concentration of iron oxide nanoparticles in the solution during the

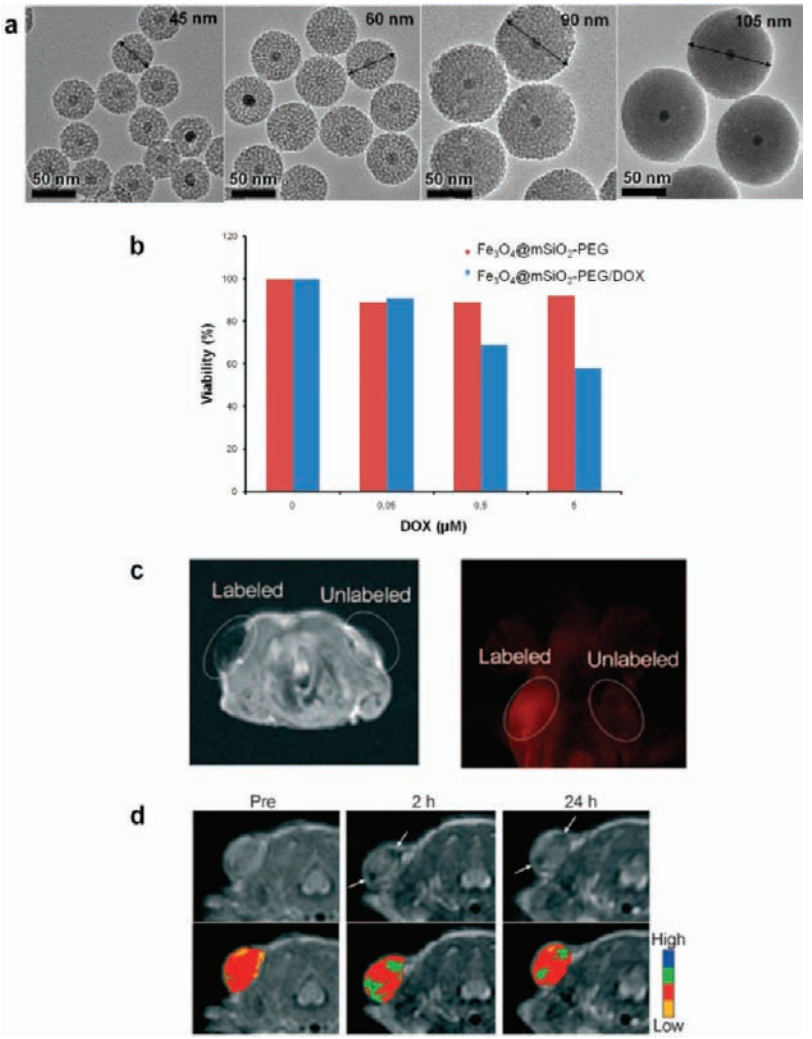


FIGURE 3. (a) Magnetite-nanoparticle/mesoporous-silica core/shell nanoparticles of various sizes (Fe_3O_4@$mSiO_2$). (b) In vitro cytotoxicity of Fe_3O_4@$mSiO_2$ and DOX-loaded Fe_3O_4@$mSiO_2$. (c) In vivo T_2-weighted MR and fluorescence images of subcutaneously injected cells labeled with Fe_3O_4@$mSiO_2$ and control cells without labeling into each dorsal shoulder of a nude mouse. (d) In vivo T_2-weighted MR images (upper row) and color maps (lower row) of the tumor before and after intravenous injection of Fe_3O_4@$mSiO_2$ to the tumor bearing nude mouse. A decrease of signal intensity on T_2-weighted MR images was detected at the tumor site (arrows). Reproduced from ref 45. Copyright 2008 Wiley-VCH.

sol–gel reaction to ensure that each silica nanoparticle contained a single nanoparticle, core/shell nanocomposite nanoparticles smaller than 100 nm with a single iron oxide nanoparticle core were obtained. The overall particle size could be controlled from 45 to 105 nm by varying the concentration of iron oxide nanoparticles (Figure 3a). The resulting core/shell nanoparticles were monodisperse and maintained discrete form without aggregation, which is highly desirable for in vivo applications.

Because Fe_3O_4@$mSiO_2$ nanocomposite nanoparticles are composed of superparamagnetic iron oxide nanoparticle core and mesoporous silica shell, they can be used not only as a magnetic resonance (MR) imaging contrast agent but also as a drug delivery vehicle. Furthermore, organic fluorescence dyes such as fluorescein isothiocyanate (FITC) and rhodamine B isothiocyanate (RITC) could be readily immobilized in mesopores, rendering them useful as fluorescent imaging probes. Surface modification with poly(ethylene glycol) (PEG) imparted biocompatibility and colloidal stability under physiological conditions. Even after PEG-coating, the hydrodynamic diameter of Fe_3O_4@$mSiO_2$ nanocomposites was kept below 100 nm and they were easily internalized into cells by endocytosis. The anticancer drug, doxorubicin (DOX), loaded nanocomposite nanoparticles were internalized by cancer cells and induced cell death (Figure 3b). The applicability of Fe_3O_4@$mSiO_2$ nanocomposite for in vivo cancer imaging was also demonstrated. Figure 3c shows in vivo MR and optical images of subcutaneously injected cancer cells labeled with

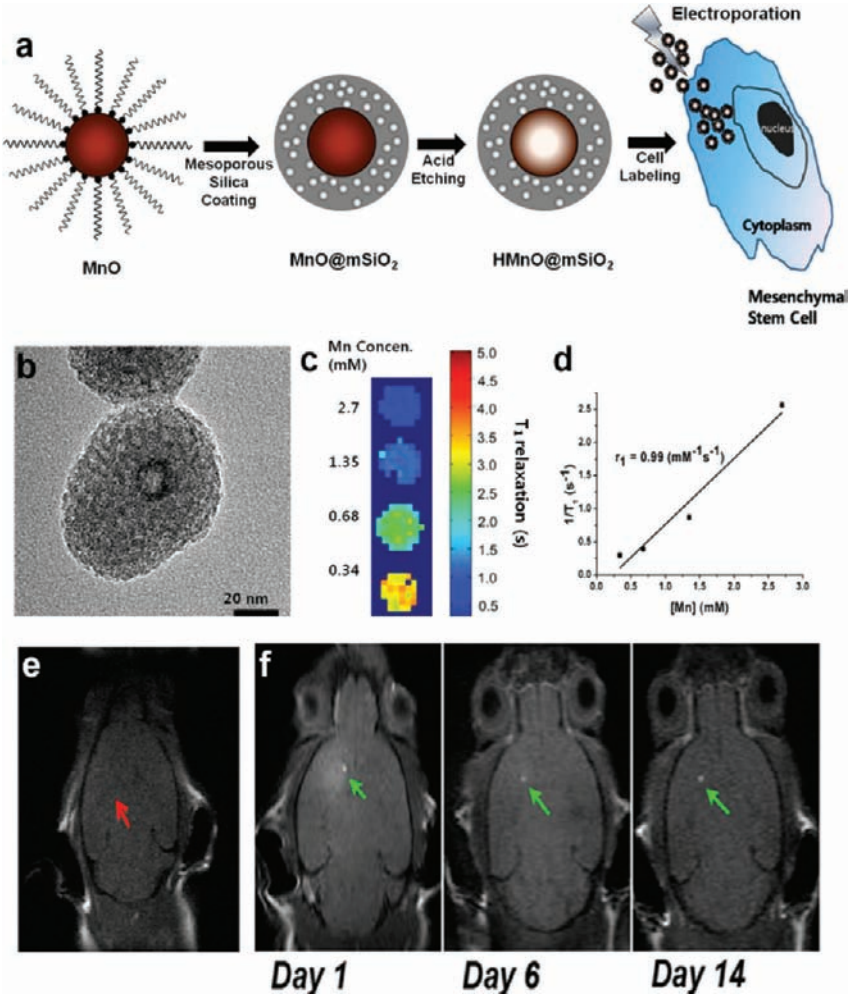


FIGURE 4. (a) Schematic illustration for synthesis of $HMnO@mSiO_2$ nanoparticles and labeling of MSCs. (b) TEM image of $HMnO@mSiO_2$ nanoparticles that shows mesoporous silica shell and hollow MnO core. (c) T_1 map of $HMnO@mSiO_2$ nanoparticles suspended in water at 11.7 T. (d) Plot of $1/T_1$ versus Mn concentration. The slope indicates the specific relaxivity (r_1). (e,f) In vivo MRI of transplanted MSCs. (e) No hyperintense signal (red arrow) was detected in mice transplanted with unlabeled MSCs. (f) Hyperintense signals (green arrows) were detected in mice transplanted with $HMnO@mSiO_2$-labeled MSCs and was still visible 14 days after injection. Reproduced from ref 50. Copyright 2011 American Chemical Society.

$Fe_3O_4@mSiO_2$. The labeled cells appeared as dark contrast in the MR image due to enhanced T_2 relaxation, and intense red emission due to RITC was also observed in the fluorescence image. Such multimodal imaging capability is very useful for both noninvasive diagnosis and guidance to surgical treatment.[47] The small size and high colloidal stability of the nanocomposite nanoparticles enabled systemic delivery to the tumor. When $Fe_3O_4@mSiO_2$ nanoparticles were administered to the tumor-bearing mice via intravenous injection into the tail vain, they were accumulated at the tumor sites and detected via MRI 2 h after the injection (Figure 3d). The multifunctional capability of the nanocomposite nanoparticles as MR and fluorescence imaging probes, along with their potential as drug delivery vehicles, makes them novel candidates for simultaneous cancer diagnosis and therapy.

Liong et al. fabricated dye-doped MSNs which were incorporated with magnetite nanoparticles and functionalized with targeting agent, folic acid, on the outer surface, for simultaneous drug delivery, magnetic resonance and fluorescence imaging, magnetic manipulation, and cell targeting.[48] The nanoparticles functionalized with folic acid exhibited more than 2-fold increase in the cellular uptake for pancreatic cancer cell lines compared to the unmodified nanoparticles. After loading of water-insoluble anticancer drug, camptothecin, into the folate-modified nanocomposite nanoparticles, considerable increase in the cytotoxicity was observed for pancreatic cancer cells, whereas cytotoxic effect on normal fibroblast cells was not changed upon addition of folate ligand. These results demonstrate that the targeting ligand enhanced cellular uptake and efficient drug delivery to the cancer cells.

Paramagnetic complexes, which are usually gadolinium (Gd^{3+}) or manganese (Mn^{2+}) chelates, accelerate longitudinal (T_1) relaxation of water protons and exert bright contrast in MR images.[39] Instead of manganese (Mn^{2+}) chelates, the Hyeon group reported the application of biocompatible manganese oxide (MnO) nanoparticles as a T_1 MRI contrast agent, which enabled easy surface modification and efficient labeling with targeting agents for their applications in molecular and cellular imaging.[49] As T_1 MR contrast agent, an increase of contact between manganese ions (Mn^{2+}) and water molecules is critical to achieve high r_1 relaxivity. For this purpose, "hollow" manganese oxide nanoparticles encapsulated with mesoporous silica shell (HMnO@$mSiO_2$) were fabricated (Figure 4a,b).[50] The mesoporous silica shell, which allows for water exchange across the shell, combined with the large surface area-to-volume ratio resulting from the novel hollow structure, increases water accessibility to the manganese core and, consequently, provides enhanced T_1 contrast. As shown in Figure 4c and d, the molar relaxivity of HMnO@$mSiO_2$ was measured to be 0.99 mM^{-1} s^{-1} at 11.7 T, which is significantly higher than that of MnO nanoparticles encapsulated with PEG-phospholipid, and dense silica-coated MnO nanoparticles. The feasibility of HMnO@$mSiO_2$ nanocomposite nanoparticles for cell labeling and tracking was demonstrated after labeling of multipotent mesenchymal stem cells (MSCs). Adipose-derived MSCs were efficiently labeled using electroporation, and signal enhancement was detected in T_1-weighted MR images. Intracranial grafting of HMnO@$mSiO_2$-labeled MSCs enabled serial MR monitoring of cell transplants over 14 days (Figure 4e,f). The results show that the HMnO@$mSiO_2$ nanocomposite nanoparticles can produce sustained contrast in vivo and, therefore, are suitable for long-term MR monitoring of the fate of the transplanted cells.

Recently, a noninvasive remote-controlled drug release system was achieved using ferrite nanoparticle-incorporated MSNs. Thomas et al. combined the hyperthermic effect of magnetic nanoparticles and controlled drug release ability of MSNs with a nanovalve system.[31] By encapsulating zinc-doped iron oxide nanoparticles within mesoporous silica shells and capping the pores with cyclic cucurbit[6]uril nanovalve, a magnetically activated release system was realized. Zinc-doped iron oxide nanocrystals (ZnNCs) offer a 4-fold increase in hyperthermic effects compared to undoped iron oxide nanocrystals. Under oscillating magnetic field, local heat was induced by incorporated magnetic nanoparticles, and this heat took off the electrostatically bound nanovalve molecules and resulted in cargo release.

2.2. Nanoparticles Assembled on Mesoporous Silica Nanoparticles. Although several kinds of nanoparticles could be encapsulated in a silica shell to fabricate multifunctional MSNs, there is still limitation on this process because encapsulation procedures are not always straightforward and easy for many kinds of nanoparticles. This weakness can be overcome by decoration of nanoparticles onto the surface of as-synthesized MSNs, which enables assembly of numerous nanoparticles and sequential addition of different functional nanoparticles.[51]

For "on-demand" controlled release of encapsulated drugs, various inorganic nanoparticles, including nanoparticles of Au, CdSe, and Fe_3O_4, were assembled on the surface of mesopores of MSNs via stimuli-responsive tethers.[32−35] These assembled nanoparticles could function as gatekeepers and could be removed by either intracellular or external triggers such as pH change, reduction process, enzyme reaction, or irradiation of light. Au nanoparticles have been most commonly used as pore capping agents because they can be easily synthesized in aqueous media and functionalized with thiol containing molecules. pH-responsive,[32] or biomolecule,[33] such as ATP, responsive release was achieved by using Au nanoparticles as capping ligands. CdS nanoparticles[34] and superparamagnetic iron oxide nanoparticles[35] have been also used as capping materials. However, these nanoparticles were used as simple caps which block the pores physically, and they do not provide any other particular function. Appropriate choice of functional nanoparticles to be bound onto MSNs can impart additional functions to MSNs for multifunctional theranostic applications.

The Hyeon group reported a simple method for assembly of hydrophobic iron oxide nanoparticles on silica particles based on covalent bonding.[52] After ligand exchange with 2-bromo-2-methylpropionic acid (BMPA), iron oxide nanocrystals were assembled on the surface of amine-functionalized silica particles through nucleophilic substitution reaction between the terminal Br groups on the surface of iron oxide nanoparticles and amino groups on the silica particles. Using this facile assembly process, a theranostic nanoplatform was achieved by addition of iron oxide nanoparticles onto MSNs. Multifunctional nanocomposite nanoparticles were fabricated by decorating the surface of dye-doped MSNs with multiple iron oxide nanoparticles (Fe_3O_4-MSN) (Figure 5a,b).[53] The integration of numerous iron oxide nanocrystals onto the silica surface is advantageous because the T_2 MR contrast effect can be further enhanced by clustering of magnetic nanoparticles.[54] Fe_3O_4-MSN showed remarkably enhanced MR contrast, and its specific relaxivity value, r_2, was increased by 2.8 times compared to

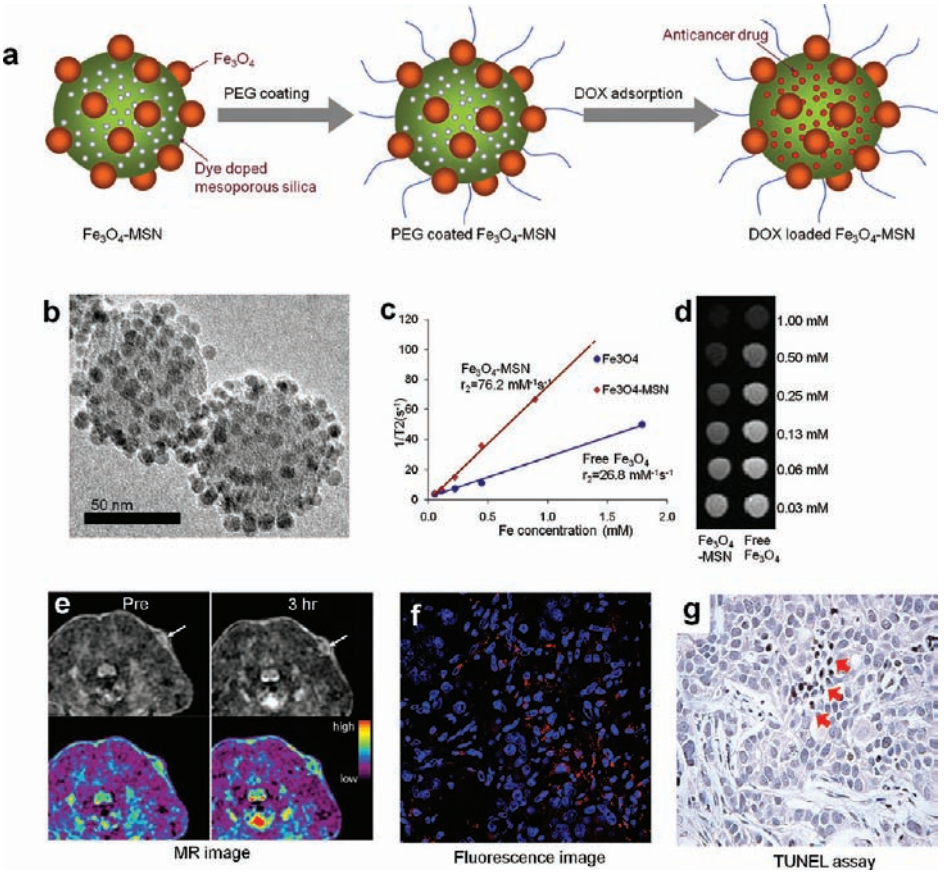


FIGURE 5. (a) Schematic illustration of the synthetic procedure for Fe_3O_4-MSN. (b) TEM image of synthesized Fe_3O_4-MSN. (c,d) T_2 contrast enhancement by Fe_3O_4-MSN. (c) Plot of inverse transverse relaxation times ($1/T_2$) versus Fe concentration. (d) T_2-weighted MR image of Fe_3O_4-MSN and free Fe_3O_4. Darker signal was observed for Fe_3O_4-MSN at the same concentration of Fe. (e) In vivo T_2-weighted MR images of the tumor site before and 3 h after intravenous injection of Fe_3O_4-MSN (arrows indicate the tumor site). (f) Confocal laser scanning microscopic image of tumor section of mouse after intravenous injection of DOX loaded Fe_3O_4−MSN. (g) TUNEL assays for apoptotic cell death. Reproduced from ref 53. Copyright 2010 American Chemical Society.

that of well-dispersed and isolated Fe_3O_4 nanoparticles (Figure 5c,d). PEG-stabilized Fe_3O_4-MSN showed excellent colloidal stability in aqueous solution and did not affect cell viability or proliferation. Accumulation of Fe_3O_4-MSN at the tumor site could be demonstrated by in vivo MR imaging after intravenous injection of Fe_3O_4-MSN into a nude mouse bearing a tumor on its shoulder. At 3 h after injection, a signal drop in the T_2-weighted MR image was clearly observed (Figure 5e), demonstrating that accumulation of Fe_3O_4-MSN at the tumor sites could be detected by in vivo MR imaging. Furthermore, Fe_3O_4-MSN could deliver therapeutic agent to the tumor sites. The red fluorescence of DOX in sectioned tumor tissues after intravenous injection of DOX-loaded Fe_3O_4-MSN allowed direct visualization of drug accumulation at the tumor sites (Figure 5f). When apoptotic cells in the tumor tissues were evaluated using terminal deoxynucleotidyl transferase-mediated nick end labeling (TUNEL) assay, a brown color, indicating TUNEL-positive tumor cell nuclei with apoptotic morphology, was detected in the tumor tissues of mice treated with DOX-loaded Fe_3O_4-MSN, thus demonstrating that DOX was successfully delivered to the tumor sites and its antitumor activity was retained (Figure 5g). Consequently, we have realized a multifunctional nanoplatform for theranostics with simultaneous imaging and therapeutic modalities that can be delivered to the tumor sites by passive targeting.

Multifunctional nanocomposite nanoparticles for simultaneous fluorescence and MR imaging, and pH-sensitive drug release were fabricated by immobilizing pH responsive hydrazone bond, magnetite nanoparticles, and fluorescent dye in MSNs (Figure 6a).[55] pH-sensitive hydrazone bond was adopted in the nanocomposite nanoparticles for pH-sensitive release of anticancer drug, DOX, because hydrazone bond is stable at neutral pH but is rapidly dissociated in acidic environments such as lysosomes, tumor sites, or infected tissues.[56] Figure 6b shows that release of DOX was both time

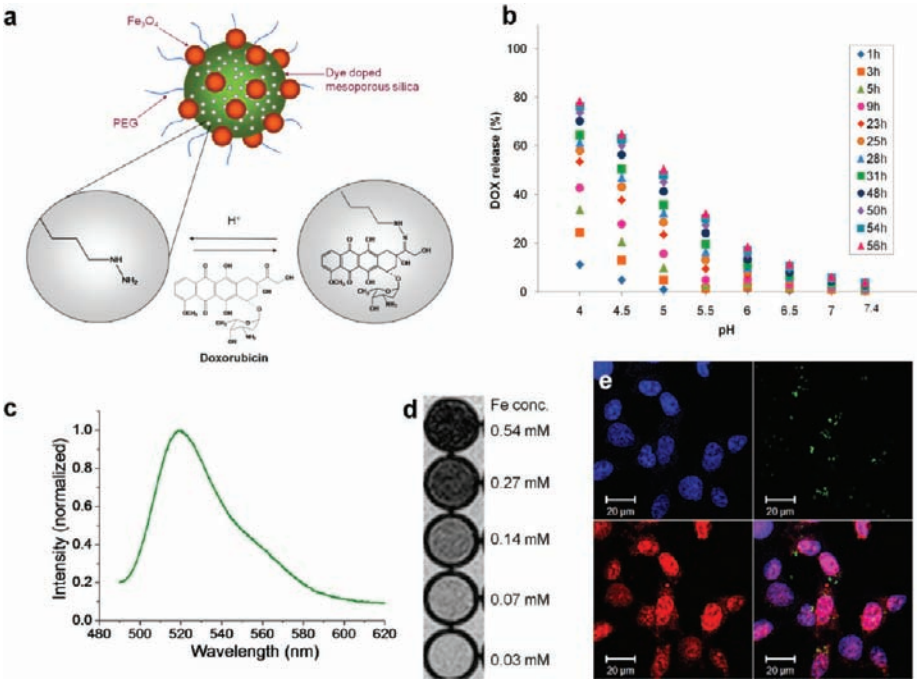


FIGURE 6. (a) Schematic illustration of hydrazine functionalized Fe_3O_4-MSN and conjugation of doxorubicin via pH-sensitive linkage. (b) Time- and pH-dependent doxorubicin release profile from hydrazine functionalized Fe_3O_4-MSN. (c) Photoluminescence spectrum and (d) T_2-weighted MR images of hydrazine-MSN-FITC-Fe_3O_4. (e) Confocal laser scanning microscopic images of cells incubated with hydrazine-MSN-FITC-Fe_3O_4-PEG for 24 h. Reproduced from ref 55. Copyright 2011 Royal Society of Chemistry.

and pH dependent. DOX was hardly released at pH 7.4, and even after 56 h only 4% of DOX was released at pH 7.4, whereas 78% of DOX was released at pH 4.0, demonstrating the pH dependent release of doxorubicin. MSNs immobilized with FITC dye and multiple magnetite nanoparticles on the surface (MSN-FITC-Fe_3O_4) displayed a typical emission peak of fluorescein at 522 nm (Figure 6c) and exhibited a high specific relaxivity value (r_2) of 80 mM^{-1} s^{-1} (Figure 6d), demonstrating that the nanocomposite nanoparticles can be used as dual imaging probe for simultaneous T_2 MRI and fluorescence imaging. Cellular uptake of DOX conjugated hydrazine-MSN-FITC-Fe_3O_4-PEG was verified by confocal laser scanning microscopy (CLSM) following the incubation of cells with the nanoparticles in serum containing cell culture media for 24 h (Figure 6e). Green fluorescence of FITC was observed in the cytoplasm and red fluorescence of DOX was found in the nucleus, clearly demonstrating that DOX was released from MSNs. The cytotoxic effect of DOX conjugated hydrazine-MSN-FITC-Fe_3O_4-PEG was tested on breast cancer cells, MDA-MB-231. The MTT assay showed that cytotoxic efficacy of the DOX conjugated MSNs increased as the concentration was increased, while bare MSNs alone did not show cytotoxicity to cancer cells even at high concentration. These results demonstrate that MSNs have a potential for drug loading and delivery into cancer cells to induce cell death.

3. Conclusion and Perspectives

In this Account, we discussed various strategies for the synthesis of multifunctional mesoporous silica nanocomposite nanoparticles as well as their applications to simultaneous therapy and diagnosis. Using MSNs as a basic building block, different kinds of functional components including magnetic nanoparticles and fluorescent dyes can be integrated to generate multifunctional nanocomposite systems while maintaining their individual functional characteristics. These nanocomposite nanoparticles were shown to be viable not only as multimodal imaging probes for simultaneous MR and fluorescence imaging but also as an anticancer drug delivery vehicle. In vivo experimental results showed that these nanoparticles were delivered to the tumor sites via passive targeting.

Although there has been explosive research activity in the development of MSN-based materials for multifunctional theranostic applications in the last several years, there remain major challenges that need to be overcome in order for these nanostructured materials to achieve translation into the clinical setting. In particular, long-term toxicity and pharmacokinectics of these MSNs should be clearly addressed. Furthermore, rigorous and extensive in vivo studies by interdisciplinary teams ranging from chemists and

materials scientists all the way to biologists and clinicians should be conducted for the clinical translations of these multifunctional theranostic agents based on mesoporous silica nanocomposite nanoparticles. If these issues are satisfactorily addressed in the future, these multifunctional nanoparticles will provide important new tools in the hands of medical doctors for simultaneous diagnosis and the efficient and specific treatment of diseases.

This work was supported by the Korean Ministry of Education, Science and Technology through Strategic Research (2010-0029138) and World Class University (R31-10013) Programs of National Research Foundation (NRF) of Korea.

BIOGRAPHICAL INFORMATION

Taeghwan Hyeon received his B.S. (1987) and M.S. (1989) in Chemistry from the Seoul National University (SNU), Korea. He obtained his Ph.D. in Chemistry from the University of Illinois at Urbana−Champaign (1996). 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, and he published more than 160 papers in prominent international journals. He is currently serving as an Associate Editor of *J. Am. Chem. Soc.* and as an editorial advisory board member of *Adv. Mater.*, *Chem. Mater.*, *Nano Today*, and *Small.*

Ji Eun Lee received her B.S. (2005), M.S. (2007), and Ph.D. (2011) from the School of Chemical and Biological Engineering (CBE) of SNU. Since 2006, she has been working on the synthesis and applications of mesoporous materials in Prof. Hyeon's laboratory.

Nohyun Lee received his B.S. (2005) and M.S. (2007) from CBE and Interdisciplinary Program in Nanoscience and Technology, respectively, of SNU, and he just received his Ph.D. from CBE of SNU. During his doctorate period, he has been working on the development of new MRI contrast agents based on magnetic nanoparticles.

Taeho Kim received his B.S. (2007) in the department of Chemical Engineering from Pohang University of Science and Technology (POSTECH) and M.S. (2010) from CBE of SNU. He is currently in the Ph.D. program working on the medical applications of multifunctional nanoparticles under the supervision of Prof. Taeghwan Hyeon.

Jaeyun Kim received his B.S. (2001), M.S. (2003), and Ph.D. (2007) from CBE of SNU under the supervision of Prof. Taeghwan Hyeon. During his graduate studies, he worked on the designed fabrication of multifunctional silica-based nanostructured materials for biomedical applications. Currently, he is working as a postdoctoral researcher at Harvard University. He received MRS Graduate Student Award in 2007.

FOOTNOTES

*To whom correspondence should be addressed. E-mail: thyeon@snu.ac.kr.

REFERENCES

1 Whitesides, G. M. The `right' size in nanobiotechnology. *Nat. Biotechnol.* **2003**, *21*, 1161–1165.

2 Katz, E.; Willner, I. Integrated nanoparticle-biomolecule hybrid systems: Synthesis, properties, and applications. *Angew. Chem., Int. Ed.* **2004**, *43*, 6042–6108.

3 Hao, R.; Xing, R. J.; Xu, Z. C.; Hou, Y. L.; Gao, S.; Sun, S. H. Synthesis, functionalization, and biomedical applications of multifunctional magnetic nanoparticles. *Adv. Mater.* **2010**, *22*, 2729–2742.

4 Chou, L. Y. T.; Ming, K.; Chan, W. C. W. Strategies for the intracellular delivery of nanoparticles. *Chem. Soc. Rev.* **2011**, *40*, 233–245.

5 Michalet, X.; Pinaud, F. F.; Bentolila, L. A.; Tsay, J. M.; Doose, S.; Li, J. J.; Sundaresan, G.; Wu, A. M.; Gambhir, S. S.; Weiss, S. Quantum dots for live cells, in vivo imaging, and diagnostics. *Science* **2005**, *307*, 538–544.

6 Medintz, I. L.; Uyeda, H. T.; Goldman, E. R.; Mattoussi, H. Quantum dot bioconjugates for imaging, labelling and sensing. *Nat. Mater.* **2005**, *4*, 435–446.

7 Rosi, N. L.; Mirkin, C. A. Nanostructures in biodiagnostics. *Chem. Rev.* **2005**, *105*, 1547–1562.

8 Jun, Y.-w.; Lee, J.-H.; Cheon, J. Chemical design of nanoparticle probes for high-performance magnetic resonance imaging. *Angew. Chem., Int. Ed.* **2008**, *47*, 5122–5135.

9 Song, H.-T.; Choi, J.-s.; Huh, Y.-M.; Kim, S.; Jun, Y.-w.; Suh, J.-S.; Cheon, J. Surface modulation of magnetic nanocrystals in the development of highly efficient magnetic resonance probes for intracellular labeling. *J. Am. Chem. Soc.* **2005**, *127*, 9992–9993.

10 Lewin, M.; Carlesso, N.; Tung, C.-H.; Tang, X.-W.; Cory, D.; Scadden, D. T.; Weissleder, R. Tat peptide-derivatized magnetic nanoparticles allow in vivo tracking and recovery of progenitor cells. *Nat. Biotechnol.* **2000**, *18*, 410–414.

11 Na, H. B.; Song, I. C.; Hyeon, T. Inorganic nanoparticles for MRI contrast agents. *Adv. Mater.* **2009**, *21*, 2133–2148.

12 Gu, H. W.; Ho, P. L.; Tsang, K. W. T.; Yu, C. Y.; Xu, B. Using biofunctional magnetic nanoparticles to capture vancomycin-resistant enterococci and other gram-positive bacteria at ultralow concentration. *J. Am. Chem. Soc.* **2003**, *125*, 15702–15703.

13 Cobley, C. M.; Chen, J. Y.; Cho, E. C.; Wang, L. V.; Xia, Y. N. Gold nanostructures: a class of multifunctional materials for biomedical applications. *Chem. Soc. Rev.* **2011**, *40*, 44–56.

14 Lal, S.; Clare, S. E.; Halas, N. J. Nanoshell-enabled photothermal cancer therapy: impending clinical impact. *Acc. Chem. Res.* **2008**, *41*, 1842–1851.

15 Gao, J.; Liang, G.; Zhang, B.; Kuang, Y.; Zhang, X.; Xu, B. FePt@CoS_2 yolk-shell nanocrystals as a potent agent to kill HeLa cells. *J. Am. Chem. Soc.* **2007**, *129*, 1428–1433.

16 Popovic, Z.; Liu, W.; Chauhan, V. P.; Lee, J.; Wong, C.; Greytak, A. B.; Insin, N.; Nocera, D. G.; Fukumura, D.; Jain, R. K.; Bawendi, M. G. A nanoparticle size series for in vivo fluorescence imaging. *Angew. Chem., Int. Ed.* **2010**, *49*, 8649–8652.

17 Peer, D.; Karp, J. M.; Hong, S.; Farokhzad, O. C.; Margalit, R.; Langer, R. Nanocarriers as an emerging platform for cancer therapy. *Nat. Nanotechnol.* **2007**, *2*, 751–760.

18 Cheon, J.; Lee, J.-H. Synergistically integrated nanoparticles as multimodal probes for nanobiotechnology. *Acc. Chem. Res.* **2008**, *41*, 1630–1640.

19 Gao, J.; Gu, H.; Xu, B. Multifunctional magnetic nanoparticles: design, synthesis, and biomedical applications. *Acc. Chem. Res.* **2009**, *42*, 1097–1107.

20 Kim, J.; Piao, Y.; Hyeon, T. Multifunctional nanostructured materials for multimodal imaging, and simultaneous imaging and therapy. *Chem. Soc. Rev.* **2009**, *38*, 372–390.

21 Piao, Y.; Burns, A.; Kim, J.; Wiesner, U.; Hyeon, T. Designed fabrication of silica-based nanostructured particle systems for nanomedicine applications. *Adv. Funct. Mater.* **2008**, *18*, 3745–3758.

22 Burns, A.; Ow, H.; Wiesner, U. Fluorescent core-shell silica nanoparticles: towards "Lab on a Particle" architectures for nanobiotechnology. *Chem. Soc. Rev.* **2006**, *35*, 1028–1042.

23 Trewyn, B. G.; Slowing, I. I.; Giri, S.; Chen, H.-T.; Lin, V. S.-Y. Synthesis and functionalization of a mesoporous silica nanoparticle based on the sol-gel process and applications in controlled release. *Acc. Chem. Res.* **2007**, *40*, 846–853.

24 Vallet-Regí, M.; Balas, F.; Arcos, D. Mesoporous materials for drug delivery. *Angew. Chem., Int. Ed.* **2007**, *46*, 7548–7558.

25 Benezra, M.; Penate-Medina, O.; Zanzonico, P. B.; Schaer, D.; Ow, H.; Burns, A.; DeStanchina, E.; Longo, V.; Herz, E.; Iyer, S.; Wolchok, J.; Larson, S. M.; Wiesner, U.; Bradbury, M. B. Multimodal silica nanoparticles are effective cancer-targeted probes in a model of human melanoma. *J. Clin. Invest.* **2011**, *121*, 2768–2780.

26 Lin, Y.-S.; Haynes, C. L. Impacts of mesoporous silica nanoparticle size, pore ordering, and pore integrity on hemolytic activity. *J. Am. Chem. Soc.* **2010**, *132*, 4834–4842.

27 Slowing, I. I.; Wu, C.-W.; Vivero-Escoto, J. L.; Lin, V. S.-Y. Mesoporous silica nanoparticles for reducing hemolytic activity towards mammalian red blood cells. *Small* **2009**, *5*, 57–62.

28 Lee, C.-H.; Cheng, S.-H.; Wang, Y.-J.; Chen, Y.-C.; Chen, N.-T.; Souris, J.; Chen, C.-T.; Mou, C.-Y.; Yang, C.-S.; Lo, L.-W. Near-infrared mesoporous silica nanoparticles for optical imaging: characterization and in vivo biodistribution. *Adv. Funct. Mater.* **2009**, *19*, 215–222.

29 Lu, J.; Liong, M.; Li, Z.; Zink, J. I.; Tamanoi, F. Biocompatibility, biodistribution, and drug-delivery efficiency of mesoporous silica nanoparticles for cancer therapy in animals. *Small* **2010**, *6*, 1794–1805.

30 Lee, C.-H.; Cheng, S.-H.; Huang, I.-P.; Souris, J. S.; Yang, C.-S.; Mou, C.-Y.; Lo, L.-W. Intracellular pH-responsive mesoporous silica nanoparticles for the controlled release of anticancer chemotherapeutics. *Angew. Chem., Int. Ed.* **2010**, *49*, 8214–8219.

31 Thomas, C. R.; Ferris, D. P.; Lee, J. H.; Choi, E.; Cho, M. H.; Kim, E. S.; Stoddart, J. F.; Shin, J. S.; Cheon, J.; Zink, J. I. Noninvasive remote-controlled release of drug molecules in vitro using magnetic actuation of mechanized nanoparticles. *J. Am. Chem. Soc.* **2010**, *132*, 10623–10625.

32 Liu, R.; Zhang, Y.; Zhao, X.; Agarwal, A.; Mueller, L. J.; Feng, P. pH-responsive nanogated ensemble based on gold-capped mesoporous silica through an acid-labile acetal linker *J. Am. Chem. Soc.* **2010**, *132*, 1500–1501.

33 Zhu, C.-L.; Lu, C.-H.; Song, X.-Y.; Yang, H.-H.; Wang, X.-R. Bioresponsive controlled release using mesoporous silica nanoparticles capped with aptamer-based molecular gate. *J. Am. Chem. Soc.* **2011**, *133*, 1278–1281.

34 Lai, C.-Y.; Trewyn, B. G.; Jeftinija, D. M.; Jeftinija, K.; Xu, S.; Jeftinija, S.; Lin, V. S.-Y. A mesoporous silica nanosphere-based carrier system with chemically removable CdS nanoparticle caps for stimuli-responsive controlled release of neurotransmitters and drug molecules. *J. Am. Chem. Soc.* **2003**, *125*, 4451–4459.

35 Giri, S.; Trewyn, B. G.; Stellmaker, M. P.; Lin, V. S.-Y Stimuli-responsive controlled-release delivery system based on mesoporous silica nanorods capped with magnetic nanoparticles. *Angew. Chem., Int. Ed.* **2005**, *44*, 5038–5044.

36 Ferris, D. P.; Zhao, Y.-L.; Khashab, N. M.; Khatib, H. A.; Stoddart, J. F.; Zink, J. I. Light-operated mechanized nanoparticles. *J. Am. Chem. Soc.* **2009**, *131*, 1686–1688.

37 Zhao, Y.-L.; Li, Z.; Kabehie, S.; Botros, Y. Y.; Stoddart, J. F.; Zink, J. I. pH-operated nanopistons on the surfaces of mesoporous silica nanoparticles. *J. Am. Chem. Soc.* **2010**, *132*, 13016–13025.

38 Meng, H. A.; Xue, M.; Xia, T. A.; Zhao, Y. L.; Tamanoi, F.; Stoddart, J. F.; Zink, J. I.; Nel, A. E. Autonomous in vitro anticancer drug release from mesoporous silica nanoparticles by pH-sensitive nanovalves. *J. Am. Chem. Soc.* **2010**, *132*, 12690–12697.

39 Taylor, K.; Kim, J.; Rieter, W.; An, H.; Lin, W. Mesoporous silica nanospheres as highly efficient MRI contrast agents. *J. Am. Chem. Soc.* **2008**, *130*, 2154–2155.

40 Vallet-Regí, M.; Colilla, M.; González, B. Medical applications of organic-inorganic hybrid materials within the field of silica-based bioceramics. *Chem. Soc. Rev.* **2011**, *40*, 596–607.

41 Vivero-Escoto, J. L.; Slowing, I. I.; Trewyn, B. G.; Lin, V. S.-Y. Mesoporous silica nanoparticles for intracellular controlled drug delivery. *Small* **2010**, *6*, 1952–1967.

42 Guerrero-Martínez, A.; Pérez-Juste, J.; Liz-Marzán, L. M. Recent progress on silica coating of nanoparticles and related nanomaterials. *Adv. Mater.* **2010**, *22*, 1182–1195.

43 Park, J.; Joo, J.; Kwon, S. G.; Jang, Y.; Hyeon, T. Synthesis of monodisperse spherical nanocrystals. *Angew. Chem., Int. Ed.* **2007**, *46*, 4630–4660.

44 Kim, J.; Lee, J. E.; Lee, J.; Yu, J. H.; Kim, B. C.; An, K.; Hwang, Y.; Shin, C.-H.; Park, J.-G.; Kim, J.; Hyeon, T. Magnetic fluorescent delivery vehicle using uniform mesoporous silica spheres embedded with monodisperse magnetic and semiconductor nanocrystals. *J. Am. Chem. Soc.* **2006**, *128*, 688–689.

45 Kim, J.; Kim, H.; Lee, N.; Kim, T.; Kim, H.; Yu, T.; Song, I.; Moon, W.; Hyeon, T. Multifunctional uniform nanoparticles composed of a magnetite nanocrystal core and a mesoporous silica shell for magnetic resonance and fluorescence imaging and for drug delivery. *Angew. Chem., Int. Ed.* **2008**, *47*, 8438–8441.

46 Perrault, S. D.; Walkey, C.; Jennings, T.; Fischer, H. C.; Chan, W. C. W. Mediating tumor targeting efficiency of nanoparticles through design. *Nano Lett.* **2009**, *9*, 1909–1915.

47 Kim, S.; Lim, Y. T.; Soltesz, E. G.; De Grand, A. M.; Lee, J.; Nakayama, A.; Parker, J. A.; Mihaljevic, T.; Laurence, R. G.; Dor, D. M.; Cohn, L. H.; Bawendi, M. G.; Frangioni, J. V. Near-infrared fluorescent type II quantum dots for sentinel lymph node mapping. *Nat. Biotechnol.* **2003**, *22*, 93–97.

48 Liong, M.; Lu, J.; Kovochich, M.; Xia, T.; Ruehm, S. G.; Nel, A. E.; Tamanoi, F.; Zink, J. I. Multifunctional inorganic nanoparticles for imaging, targeting, and drug delivery. *ACS Nano* **2008**, *2*, 889–896.

49 Na, H. B.; Lee, J. H.; An, K.; Park, Y. I.; Park, M.; Lee, I. S.; Nam, D.-H.; Kim, S. T.; Kim, S.-H.; Kim, S.-W.; Lim, K.-H.; Kim, K.-S.; Kim, S.-O.; Hyeon, T. Development of a T_1 contrast agent for magnetic resonance imaging using MnO nanoparticles. *Angew. Chem., Int. Ed.* **2007**, *46*, 5397–5401.

50 Kim, T.; Momin, E.; Choi, J.; Yuan, K.; Zaidi, H.; Kim, J.; Park, M.; Lee, N.; McMahon, M. T.; Quinones-Hinojosa, A.; Bulte, J. W. M.; Hyeon, T.; Gilad, A. A. Mesoporous silica-coated hollow manganese oxide nanoparticles as positive T_1 contrast agents for labeling and MRI tracking of adipose-derived mesenchymal stem cells. *J. Am. Chem. Soc.* **2011**, *133*, 2955–2961.

51 Caruso, F. Nanoengineering of Particle Surfaces. *Adv. Funct. Mater.* **2008**, *18*, 3745–3758.

52 Kim, J.; Lee, J. E.; Lee, J.; Jang, Y.; Kim, S.-W.; An, K.; Yu, J. H.; Hyeon, T. Generalized fabrication of multifunctional nanoparticle assemblies on silica spheres. *Angew. Chem., Int. Ed.* **2006**, *45*, 4789–4793.

53 Lee, J. E.; Lee, N.; Kim, H.; Kim, J.; Choi, S. H.; Kim, J. H.; Kim, T.; Song, I.; Park, S. P.; Moon, W.; Hyeon, T. Uniform mesoporous dye-doped silica nanoparticles decorated with multiple magnetite nanocrystals for simultaneous enhanced magnetic resonance imaging, fluorescence imaging, and drug delivery. *J. Am. Chem. Soc.* **2010**, *132*, 552–557.

54 Perez, J.; Josephson, L.; O'Loughlin, T.; Högemann, D.; Weissleder, R. Magnetic relaxation switches capable of sensing molecular interactions. *Nat. Biotechnol.* **2002**, *20*, 816–820.

55 Lee, J. E.; Lee, D. J.; Lee, N.; Kim, B. H.; Choi, S. H.; Hyeon, T. Multifunctional mesoporous silica nanocomposite nanoparticles for pH controlled drug release and dual modal imaging. *J. Mater. Chem.* 2011, published online June 24, DOI: 10.1039/C1JM11869B.

56 Kalia, J.; Raines, R. T. Hydrolytic stability of hydrazones and oximes. *Angew. Chem., Int. Ed.* **2008**, *47*, 7523–7526.