1. Introduction

Hollow nanostructured materials with well-defined morphologies have attracted considerable interest because of their unique properties, including their low density, large surface area, and high guest-loading capacity, resulting in the corresponding broad application potentials in catalysis, nanoreactors, and drug/gene delivery for biomedical imaging/therapy.[1–6] Among various hollow nanomaterials, hollow silica nanocapsules (HSNs), especially with porous shells, are of great significance in nanobiomedical fields, largely due to their large surface area, high pore volume, tunable pore sizes, and excellent biocompatibility.[7] HSNs with small mesopores (2–3 nm) in the shell have been demonstrated as excellent drug-delivery systems (DDSs) both in vitro and in vivo.[8–13] However, there still exists a technical limitation using HSNs to encapsulate and deliver large entities to satisfy varied prerequisites, such as bio-macromolecules (e.g., siRNA or DNA) or nanoparticles (NPs) because it is currently still a big challenge to synthesize HSNs with well-defined and large enough pore channels (>10 nm) in the shell, while the diffusion of large molecules or NPs through the shells with pores <10 nm is usually a slow process or even impossible.[14]

Pore sizes of bulk mesoporous materials, synthesized by the traditional surfactant structure-directing strategies, can be tuned on a relatively large scale.[15–21] However, such a
Surfactant-assisted synthesis contributes little to the fabrication of large-sized porous materials of nanosized particles with well-defined morphologies, not to mention those with hollow nanostructures. Recently, great efforts have been devoted to synthesizing hollow silica microparticles with large pores in the shell via various special approaches. Cagelike hollow aluminosilicates with vermiculate micro-through-holes were successfully prepared by Shiomi et al. by using lysozyme as the pore-making agent, and their use for the ship-in-a-bottle encapsulation of proteins has been explored. By adding water-soluble polymers to a water/oil double-emulsion system, Fujiwara et al. fabricated diatomaceouslike hollow silica spheres with nano-macroholes. Li et al. reported cagelike hollow silica spheres loaded with superparamagnetic iron oxide NPs in their macroporous shells by a one-step oil-in-diethylene glycol microemulsion route. Recently, layers of silica with macroholes were produced on PS substrates by controlled silica-seed coalescence, which were further employed as the nanoparticle collectors. However, the particle sizes of reported materials, usually in the micrometer size range, are too large to be applied in nanobiomedical applications, in addition to their irregular morphologies, nonuniform size distributions, and large aggregates. For instance, the cut-off size of the permeabilized vascular tumor tissues varies in the range 200–800 nm. The DDSs or nanoprobes of particle sizes in such a range (or smaller) may be used to ensure high efficacy of enhanced permeability and retention (EPR)-mediated drug delivery or biological imaging for cancer therapy and diagnosis. Unfortunately, HSNs of small sizes (e.g., <500 nm) can usually only be fabricated with small pore sizes (e.g., <5 nm) in the shell. Therefore, the preparation of HSNs with both small particle sizes and simultaneously large enough pore diameters is still a great challenge.

As an important branch of chemistry, sol–gel chemistry has contributed greatly to the fabrication of inorganic materials such as glasses and ceramics with tunable compositions, structures, morphologies, and properties. Typically, the sol–gel process is based on the hydrolysis and condensation of alkoxides under acidic or alkaline conditions. It is worth noting that this chemical reaction is a reversible process, which is illustrated in Scheme 1. Importantly, this reversible reaction provides opportunities to design and fabricate nanostructured materials with desired structures and functionalities by the elaborative control over the processing technology.

Herein, we describe the modulation of pore sizes in the shell of HSNs based on this special reversible chemical process. The pore sizes can be tuned beyond 10 nm by the Si–O bond-breaking process, while the pore sizes can also be reduced to zero by the Si–O bond reformation. Basically, a general surfactant-directing alkaline etching strategy (SDAE) has been developed to successfully enlarge the pore sizes in the shells of HSNs. This synthetic strategy is very simple, scalable, and economic, and needs only the treatment of as-synthesized NPs in alkaline solutions for selected time intervals and/or etchant concentrations. The large pores in the silica shell are well-defined, and the particle sizes are smaller than 300 nm, which were further explored for encapsulation and intracellular transfection of siRNA molecules. In addition, small-sized superparamagnetic Fe₃O₄ NPs were captured by the large-sized pore HSNs by a vacuum-impregnation technique. The superparamagnetic properties of the fabricated composite NPs and their in-vitro T₂-weighted MR imaging capabilities were also demonstrated.

2. Results and Discussion

2.1. Design, Synthesis, and Characterization of Monodispersed and Nanosized HSNs

The procedure for tuning the pore sizes in the shell of HSNs by the reversible Si–O bond breakage and reformation is shown in Scheme 2. Solid silica core/mesoporous silica shell NPs (denoted as sSiO₂@mSiO₂) were firstly synthesized by the cocondensation of tetraethyl orthosilicate (TEOS) and octadecyltrimethoxysilane (C₁₈TMS) to form a mesoporous silica layer on the surface of Stöber-based silica NPs. Our previous results have demonstrated that the solid silica core could be selectively etched away by a special structural difference-based selective-etching strategy based on the differences in condensation/densification degrees between the core and shell, leaving highly dispersed small pore-sized hollow mesoporous silica nanocapsules (step 1).

Considering the unstable nature of Si–O bond under alkaline conditions, a novel synthetic strategy is proposed to tune the pore sizes in the shell of HSNs: a surfactant-directing alkaline etching (SDAE) process. In HSNs, the small pores generated from self-assembled micelles of surfactants could fuse with each other to create larger pores by the breakage of
Si–O bonds in between the small pores on the wall (step 2). From a chemical point of view, the large pores in the shell of HSNs are generated by two things; one is the micelles, which are formed by the self-assembly of surfactants, while the other is the controllable alkaline etching of the shell, leading to the partial breakage of Si–O bonds in the shell and consequently the expanded pore diameters. In the meantime, the regrowth process occurs by the condensation of dissolved silicate species with silanols (Si–OH) in the pore surfaces, such as monomeric silicic acid and various polysilicic acids of different polymerization degrees (step 3).\textsuperscript{[28,29]} If the regrowth rate becomes faster than the dissolution process, the pores could be partially or completely filled to form HSNs without pores (step 4).

The transmission electron microscopy (TEM) image of sSiO\textsubscript{2}@mSiO\textsubscript{2} NPs (Figure 1a) shows highly uniform and monodispersed spheres with distinctive core/shell nanostructures. The magnified TEM image (inset of Figure 1a) exhibits that the pores in the mesoporous silica shell are randomly distributed over the mesoporous shell and the core is solid and nonporous (Supporting Information (SI), Figure S1,S2). After the treatment of sSiO\textsubscript{2}@mSiO\textsubscript{2} NPs in Na\textsubscript{2}CO\textsubscript{3} solution at 80 °C (0.6 M, 50 mL) for 4 h, the solid silica core is completely etched away to form the hollow structure, which is evidenced by the clear contrast difference in TEM image (Figure 1b and inset). To investigate the influence of etching time for the modulation of pore sizes, different etching time intervals were applied. TEM images of HSNs with various etching time intervals (4, 5, 7, 10, and 24 h) were acquired to directly observe the evolution of pore structures. As shown in Figure 1b–f, the spherical morphology of HSNs can be well-preserved even etched for as long as 24 h. Interestingly, the pores in the shell are effectively enlarged at increased etching time of up to 10 h, as can be identified in TEM images (Figure 1e), by simply monitoring the etching process.

The N\textsubscript{2} adsorption–desorption technique provides direct evidence of the pore structural evolutions during the etching process. As shown in Figure 2a, the sSiO\textsubscript{2}@mSiO\textsubscript{2} NPs and HSNs achieved by different etching times exhibit typical Langmuir IV hysteresis loops, indicating the existence of well-defined mesopores. Figure 2a shows that longer etching times will result in higher relative pressures at which the pressure leaps occur, demonstrating that the pore sizes have been expanded by prolonging the etching time. Calculated by the typical Barrett–Joyner–Halenda (BJH) method, the average pore sizes of sSiO\textsubscript{2}@mSiO\textsubscript{2} and HSNs by etching for 4, 5, 7, and 10 h are 2.6, 3.2, 6.4, 12.6, and 14.6 nm, respectively (Figure 2b). With the etching time extended to 24 h, TEM images of HSNs show that spherical morphology could be still preserved and larger pores could be formed to generate the cagelike structure (Figure 1f). However, it is also very interesting to observe from TEM images (Figure 3a,b) that some of the pores on spherical NPs were fully filled by the regrowth of dissolved silicate species to form nearly nonporous shells, demonstrating that the regrowth process took place simultaneously during etching, which was further
confirmed by N$_2$ adsorption–desorption isotherm measurements (Figure 3c,d).

Importantly, the fully nonporous HSNs could be synthesized when the etchant (Na$_2$CO$_3$) amounts were increased. We adopted two separate routes to increase the etchant (Na$_2$CO$_3$) amount: one was to raise etchant volume while its concentrations and time were kept constant (Na$_2$CO$_3$ aqueous solution: 80 mL, 0.6 M), and the other was to increase the etchant concentration while the etchant volume and etching time remained unchanged (Na$_2$CO$_3$ aqueous solution: 50 mL, 1.2 M). Interestingly, the HSNs become entirely nonporous in a short etching period (4 h), as can be seen from the TEM images (Figure 4a$_1$,a$_2$ and Figure 4b$_1$,b$_2$). The spherical morphology of HSNs has been well preserved. The nonporous structures were further confirmed by N$_2$ adsorption–desorption isotherm measurements. Figure 5a shows the absence of the representative hysteresis loop for porous structures, demonstrating the nonporous structure in HSNs above mentioned. The corresponding pore-size distribution curves further confirm their nonporous structure (Figure 5b).

The above TEM observations and N$_2$ adsorption–desorption characterizations render us to conceive the mechanism of pore size evolution in the shell of HSNs. In general, the Si–O bond breakage by coordinating OH$^-$ to Si atoms, and meanwhile the bond reformation by dehydration between silanols (Si–OH) can take place simultaneously, which are based on the typical reversible alkoxides hydrolysis and condensation reaction (Scheme 1).[30] The initial etching stage takes the advantage of the structural differences between the core and shell of sSiO$_2$@mSiO$_2$, resulting in the dissolution of the cores.[8] As the etching continues, more and more dissolved products such as monomeric silicic acid and various polysilicic acids with different polymerization degrees are generated and enter the etching solutions.[28,29] Meanwhile, the increased concentrations of silicate species will accelerate the chemical reaction between the Si–OH groups on the pore walls and dissolved silicate species in aqueous solution, initiating the regrowth process of the silica wall. When the regrowth becomes faster than dissolution, the pores in the shell could be gradually filled, and finally be completely filled to form HSNs without pores.

Figure 2. N$_2$ adsorption–desorption isotherms and the corresponding pore-size distributions of sSiO$_2$@mSiO$_2$ and HSNs after different etching time (0, 4, 5, 7, and 10 h).

Figure 3. a,b) Selected TEM image of HSNs after 24 h etching (0.6 M Na$_2$CO$_3$ aqueous solution, 50 mL) for the direct observation of the cagelike pore structure and regrowth of dissolved silicate species in the shell of HSNs; N$_2$ adsorption–desorption isotherm (c) and the corresponding pore size distributions (d) of HSNs after 24 h etching.
2.2. Large-Pore Sized HSNs for siRNA Delivery and Intracellular Transfection

RNA interference (RNAi) has become an effective and promising technique to knockdown specific gene expression for gene therapy since it was first discovered in 1998. One of the biggest challenges in the progress of the RNAi technique is how to deliver the exogenous double-stranded siRNA into the cytoplasm of the target cells efficiently, and thus to trigger the RNAi effect. Because the naked siRNA can hardly penetrate the cell membrane of the target cells through passive diffusion, application of nanocarriers for siRNA delivery is vitally important for effective siRNA delivery. Porous silica NPs have served as the nonviral gene-transfection carrier for siRNA delivery and intracellular transfection. However, the pore sizes of reported silica NPs were too small to load siRNA in the pores, and siRNA molecules were usually present on the surface of NPs.

Comparatively, the encapsulation of siRNA molecules into the pores could combine the merits of resistance to RNAse degradation and sustained siRNA release from the pores, where the pores truly play roles. It is known that cationic polyelectrolyte polyethyleneimine (PEI) is an efficient agent in gene delivery because of the so-called ‘proton sponge’ effect for its strong escape ability from the endosomes in cells. Therefore, the inner pore surface of large-sized pore HSNs was modified by the PEI molecules of different molecular weights ($M_w$ = 600, 1800, 10 000, and 25 000) to make the surface of pores positively charged. Zeta potentials of HSNs and PEI–HSNs after different PEI modifications are $-32.6 \pm 1.3$ mV, $29.1 \pm 1.5$ mV ($M_w$ = 600), $36.3 \pm 2.4$ mV ($M_w$ = 1800), $27.8 \pm 3.8$ mV ($M_w$ = 10 000), and $38.5 \pm 3.0$ mV ($M_w$ = 25 000), respectively (Figure 6a).

Though PEI–HSNs with PEI $M_w$ = 25 000 possess the highest zeta potential, the high cytotoxicity of high-$M_w$ PEI molecules hinders their further biological application. In this work, PEI with moderate $M_w$ (1800) and relatively high zeta potential for further modifications and subsequent assessments. The positively charged PEI–HSNs NPs can bind negatively charged siRNA through electrostatic attractions (Figure 6a). After the impregnation of siRNA into PEI–HSNs, the amount of free siRNA left in the supernatant was determined to assess their loading capacity. The results show that the free siRNA left in the supernatant decreased quickly with increasing the weight ratio (WR) of PEI–HSNs to siRNA (Figure 6c). The capability of PEI–HSNs to encapsulate siRNA was further confirmed by gel electrophoresis. The band remained bright at WR = 9 while it completely disappeared when the WR increased to 21 (Figure 6d), indicating that the negatively charged siRNA could be entirely neutralized and encapsulated into HSNs at this WR value.

![Figure 4](image4.png) TEM images of HSNs with nonporous shells after the treatment in $a_1,a_2$ 80 mL Na$_2$CO$_3$ (0.6 w) or $b_1,b_2$ 50 mL Na$_2$CO$_3$ (1.2 w) aqueous solutions for 4 h.

![Figure 5](image5.png) $N_2$ adsorption–desorption isotherms (a) and the corresponding pore-size distributions (b) of nonporous HSNs after etching in 0.6 M Na$_2$CO$_3$, 80 mL for 4 h and 1.2 M Na$_2$CO$_3$, 50 mL for 4 h.
shown in Figure 7a,b, a large number of PEI–HSN NPs could be found in the cytoplasm, and their hollow nanostructure was still maintained. The possible uptake mechanism is revealed by selected cell domains where the NPs are present. Part of PEI–HSN NPs are found near the cell membrane to form vesicles (region A in Figure 7a) and the formed vesicle can be observed in region B (Figure 7a). The PEI–HSNs located in endosomes and their escape from endosomes can also be observed in region C (Figure 7b).41,42 The efficient uptake of PEI–HSN NPs by cells guarantees siRNA transfection efficiency. The effect of siRNA in reducing HEK293T mRNA and protein expressions was analyzed using the typical western blot analysis. The expression of GAPDH protein could be down-regulated by siRNA targeted to HEK293T. A comparison of transfection efficiency between negative control siRNA (NCsiRNA) and PEI–HSNs/siRNA are shown in Figure 7c,d. The lipofectamine 2000-mediated RNAi was used as the positive control and β-actin was employed for determining the same siRNA amount used in all the experiment. The results show that the expression of GAPDH protein is significantly inhibited by PEI–HSNs/siRNA (64%), demonstrating the efficient siRNA transfection by PEI–HSNs, while the NCsiRNA exhibit no obvious protein-expression inhibition effect (6% in Figure 7c,d). The transfection efficiency of PEI–HSNs is comparable to the commercial lipofectamine 2000, though the efficiency is a little lower.

2.3. Large-Pore Sized HSNs as Nanoparticle Collectors

It has been demonstrated that drug molecules could be encapsulated into mesopores when the molecular sizes of the drugs are smaller than the pore sizes.40,43,44 Comparatively, it is anticipated that NPs could also be loaded into pores, provided that the particle sizes of NPs are smaller than the pore sizes.14 To demonstrate this assumption, we chose the typical hydrophobic superparamagnetic iron oxide nanoparticles (denoted as SPIONs) of ~5 nm in diameter to be loaded into the large-sized pore HSNs. In addition, we adopted a vacuum-impregnation technique (Figure 8a) to enhance the loading amount of SPIONs into the silica nanocapsules.45 Different initial SPION concentrations in hexane solutions (0.5, 1, and 2 mg mL⁻¹) were employed to assess the capturing amount and efficiency of HSNs for SPIONs. As shown in Figure 8b, almost all SPIONs can be loaded into HSNs, implying a near-100% collecting efficiency for the initial SPION concentration of 0.5 mg mL⁻¹, as visually observed from the absence of SPION color in hexane (Figure 8b). Importantly, the achieved SPION–HSNs exhibit...
Reversible Pore-Structure Evolution in Hollow Silica Nanocapsules

A hydrophilic nature, as demonstrated by their excellent dispersity in aqueous solution (Figure 8c), which is due to the hydrophilic nature of the silica nanocapsules. When an external magnetic field was applied, the SPION–HSNs were attracted towards the magnet and the aqueous solution became clear and transparent (Figure 8c), suggesting the feasibility of SPION–HSN composite NPs to be manipulated by an external magnetic field. As shown in Figure 8d, monodisperse SPIONs with particle sizes of about 5 nm could be obtained by a novel green hydrothermal synthesis using iron stearic acid (Fe(SA)3) as the iron precursor and 1,2-dodecanediol as the reducer, and the SPIONs are well-crystallized as can be seen from the high-resolution TEM characterization (Figure 8e). TEM images (Figure 8f,g) of SPION–HSNs show numerous black dots distributed uniformly in the shell, demonstrating that SPIONs could be readily collected within the pores of HSNs.

Traditional strategy towards multifunctionalization of silica-based materials is a coating process, including surface modification of multifunctional nanocrystals and subsequent silica-layer coating to obtain core/shell nanostructure with multifunctional nanocrystals as the core and silica as the shell. However, the fabrication of the core/shell nanostructure is time-consuming and needs a harsh synthetic environment, which greatly limits their scalable synthesis. Here a vacuum-impregnation process was employed to introduce the SPIONs into silica nanocapsules, in contrast to the traditional coating process by transforming hydrophobic NPs from an organic phase to an aqueous solution and the subsequent silica-coating process. The achieved SPION–HSNs exhibit superparamagnetic properties from the absence of magnetic hysteresis loops (Figure 9a). The saturation magnetizations for SPION–HSNs-n (n = 1, 2, or 3) are 3.36 emu g⁻¹ (SPION–HSNs-1), 4.69 emu g⁻¹ (SPION–HSNs-2) and 5.17 emu g⁻¹ (SPION–HSNs-3). These superparamagnetic composite NPs could be used in separation, catalysis, enzyme immobilization, and nanomedicine. The SPIONs in HSNs have been specially applied as the contrast agents for magnetic resonance imaging (MRI), which was demonstrated by the decreased transverse relaxation time (T2) of SPION–HSNs, as can be seen from the changes of signal intensity with the increasing of iron concentrations (images inset of Figure 9b). The T2 value is determined to be 45.0 ms⁻¹ by the curve of transverse relaxation rate (1/T2) as a function of the iron concentration (Figure 9b). It is believed that other functional NPs could also be captured by the large-sized pore HSNs, provided that the particle sizes of the NPs are smaller than the pore sizes of HSNs.

3. Conclusion

In summary, we report here an efficient surfactant-directing alkaline-etching strategy to tune the pore sizes in the shells of hollow silica nanocapsules, based on a reversible alkoxide dissolution/recondensation chemical process. The pores can be tuned from 3.2 nm to larger than 10 nm by monitoring the etching process, while the pore channels can also be re-filled by increasing the etchant amount to form non-porous hollow silica nanocapsules. The large-sized pore HSNs were employed to encapsulate large entities such as biomolecules (siRNA) and nanoparticles (Fe₃O₄ NPs). The results show that surface-functionalized HSNs could effectively encapsulate siRNA molecules and a high siRNA transfection...
4. Experimental Section

**Materials**: Tetraethyl orthosilicate (TEOS), ethanol (EtOH), 
Na$_2$CO$_3$, ammonia solution (25–28%) and polyvinyl pyrrolidone (PVP, K30) were purchased from Sinopharm Chemical Reagent Co. Octadecytrimethoxysilane (designated as C$_{18}$TMS), iron stearic acid (Fe(SA)$_3$, 0.9 g), 1,2-dodecanediol (0.6 g) and PVP (K30, 1.0 g) were dispersed into ethanol (70 mL) by magnetically stirring for 3 h at room temperature. Subsequently, the solution was transferred into a Teflon-lined autoclave (100 mL capacity), then being sealed and heat-treated at 150 °C for another 24 h. After the autoclave was cooled down to room temperature, the precipitate was washed with plenty of ethanol and acetone several times and dried under vacuum at room temperature overnight for further use.

**Vacuum-Impregnation of SPIONs into HSNs**: HSN powder (100 mg) was placed in the bottom of a glass vessel and then subjected to vacuum under the ultrasonic treatment (Apparatus, see Figure 8a). After about 20 min, the vacuum pump was turned off. The SPIONs in hexane solution (20 mL; 0.5, 1, and 2 mg mL$^{-1}$) was added from the top of the glass vessel dropwise. This technique principle is based on the pressure differences between the outer and inner vessel that facilitate the impregnation of substances into the pores. Then, the vacuum pump was turned on. The process was lasted for another 5 min. Finally, the air was filled into the glass vessel to balance the inner and outer atmosphere. The SPION–HSN NPs were obtained by centrifugation and washed by hexane for several times. The products were dried under vacuum and calcined at 550 °C for 6 h to remove the surfactants (C$_{18}$TMS).

**Synthesis of Nonporous HSNs**: Two separate routes were adopted to achieve the nonporous HSNs, both of which were all based on the increase of the etchant amount during etching process. Typically, the prepared sSiO$_2$@mSiO$_2$ NPs were dispersed into 0.6 m Na$_2$CO$_3$ aqueous solution (80 mL) or 1.2 m Na$_2$CO$_3$ aqueous solution (50 mL), which were further stirred for another 4 h. The subsequent treatment was the same for large-pore size HSNs.

**Synthesis of Nonporous HSNs**: Two separate routes were adopted to achieve the nonporous HSNs, both of which were all based on the increase of the etchant amount during etching process. Typically, the prepared sSiO$_2$@mSiO$_2$ NPs were dispersed into 0.6 m Na$_2$CO$_3$ aqueous solution (80 mL) or 1.2 m Na$_2$CO$_3$ aqueous solution (50 mL), which were further stirred for another 4 h. The subsequent treatment was the same for large-pore size HSNs.

Efficiency can be achieved. The Fe$_3$O$_4$-loaded HSNs exhibit superparamagnetic properties, and their use as T$_2$-weighted MRI contrast agents was demonstrated by giving a signal-decreasing effect using a clinical MRI apparatus. As the sizes of many more entities (molecules or NPs) are within the pore-size ranges of as-prepared HSNs, the present HSNs of varied pore sizes will make it highly possible to encapsulate and deliver them to satisfy various requirements in applied fields such as separation, catalysis, nanobiomedicine, etc.
**Preparation of PEI–HSN/siRNA NPs:** PEI–HSN NPs were dispersed in physiological saline (pH = 7.6) with the concentration of 3 mg mL\(^{-1}\) as the stock solution. According to various N/P ratios (Weight Ratio (WR) was used here to simplify the calculation), appropriate volumes of siRNA (20 µl) and PEI–HSNs stock solutions were added to DEPC water. Then it was vortexed 30 s, 3 times and incubated for 12 h at room temperature to induce PEI–HSN/siRNA nanocomplex formation.

**siRNA-Loading Efficiency Assay:** After incubation for 12 h, the suspension was centrifuged for 30 s at 6000 rpm to separate the supernatant and PEI–HSN/siRNA nanocomplex. siRNA concentration in the supernatant was analyzed using the Nanodrop instrument (Thermo, USA). Gel electrophoresis was performed to evaluate siRNA leftovers in the supernatant and siRNA condensation. PEI–HSN/siRNA NPs were prepared with different WRs, and the complexes were loaded onto 15% polyacrylamide gel electrophoresis (PAGE) gels with 6× loading buffer (1 µl). The mixture was separated in 0.5×Tris/borate/ethylenediamine tetraacetic acid (EDTA) buffer (TBE) at 100 V for 30 min. siRNA bands were visualized using ImageQuant 300 (GE).

**Bio-TEM:** The HEK293T cells were co-incubated with 100 µg mL\(^{-1}\) PEI–HSN in DMEM medium in 5% CO\(_2\) at 37°C for 24 h. Afterwards, the cells were washed twice with cold PBS (pH = 7.4) to remove the NPs not taken up and detached by incubation with 0.25% trypsin for 5 min. The cell suspension was further centrifuged at 5000 × g min\(^{-1}\) for 2 min. After removing the incubation media, the cells were fixed by glutaraldehyde at room temperature. For resin embedding, the cell sample was rinsed with PB and dehydrated through a graded ethanol series, then cleared with propylene oxide. The samples were then embedded in EPOSM12 and polymerized in the oven at 37°C for 12 h, and 60°C for 48 h. Ultrathin sections of approximately 70 nm thick were cut for resin embedding. The cell sample was then fixed on a copper grid. Finally, the TEM images were viewed on JEM-1230 electron microscope.

**Cell Transfection:** HEK293T cells were seeded into 6-well plates at a density of 10\(^4\) cells per well for 24 h. PEI–HSN/siRNA complexes were prepared according to the different weight ratios. The PEI–HSNs/siRNA complexes were gently mixed with cell culture medium at a final volume of 2 mL per well and incubated for 10–15 min at room temperature. The original cell culture media was replaced with media containing PEI–HSN/siRNA. Cells were then incubated in the transfection media for 72 h without changing the media. GAPDH targeted siRNA sequences were outlined as follows: sense strand: 5′-GUA UGA CAA CAG CCU CAA GTT dtdt-3′; and antisense strand: 5′-CUU GAG GCU GUC AUA CTT dtdt-3′. The negative control siRNA sequences were outlined as follows: sense strand: 5′-UUC UCC GAA CGU GUC AGC UTT dtdt-3′; and antisense strand: 5′-ACG UGA CAC GUU CGG AGA ATAT dtdt-3′.

**Western-Blot Analysis:** After incubation for 72 h, total protein was extracted in RIPA buffer (Beyotime, Shanghai, China) with protease inhibitors (PMSF). Protein concentrations were measured using Bio-Rad DC Protein Assay Kit. Equal amounts of total proteins were separated in 8% sodium dodecyl sulfate (SDS) polyacrylamide gel and transferred to polyvinylidene (PVDF) membranes. The membranes were blocked in 5% nonfat dry milk in Tris-buffered saline tween-20 (TBST) for 2 h at room temperature and incubated with mouse anti-GAPDH antibodies (1:1000) overnight at 4°C. After three washes with TBST, membranes were incubated with anti-mouse IgG-HRP for 1 h at room temperature. Enhanced chemiluminescence (ECL) detection reagent (GE) was used, and the protein bands were visualized after exposure with X-ray film. β-actin expression was used as the housekeeping gene control. Images analyses were performed with ImageQuant 300.

**MRI test:** The in-vitro MR imaging experiment was carried out on a 3 T clinical MRI instrument (GE Sigma 3.0 T, Shanghai Cancer Hospital), and the pulse sequence employed was a T\(_2\)-weighted Fast-recovery fast spin-echo (FR-FSE) sequence with the following parameters: TR = 4000 ms, slice thickness = 3.0 mm, echo time = 15 ms. Before MRI test, the Fe concentrations of PEI–HSNs in aqueous solution were determined by inductively coupled plasma atomic emission spectrometry (ICP-AES).

**Characterization:** Transmission electron microscopy (TEM) images were acquired on a JEM-2100F electron microscope operating at an accelerating voltage of 200 kV. N\(_2\) adsorption–desorption isotherms at 77 K were measured on a Micrometics Tristar 3000 system. All samples were pretreated for 12 h at 393 K under N\(_2\) flow before measurements. The pore size distributions were calculated by the Barrett–Joyner–Halenda (BJH) method. The pore volume and surface area were calculated by using typical Barrett–Joyner–Halenda (BJH) and Langmuir methods, respectively. The magnetic properties of SPION–HSNs were characterized by a vibrating magnetometer (PPMS Model 6000 Quantum Design). X-ray diffraction (XRD) patterns were collected using a RigakuD/Max-2200 PC X-ray diffractometer with Cu target (40 kV, 40 mA). Field emission scanning electron microscopy (FESEM) images were obtained on a field emission HITACHI S-4800 microscope.

**Supporting Information**
Supporting Information is available from the Wiley Online Library or from the author.

**Acknowledgements**

This work was supported by the National Basic Research Program of China (973 Program, Grant No.2011CB707905), Shanghai Rising-Star Program (10QH1402800), National Nature Science Foundation of China (Grant No. 50823007, 51072212 and 30971089), the Science Foundation for Youth Scholar of State Key Laboratory of High Performance Ceramics and Superfine Microstructures (Grant No. SKL201001) and the Chinese Academy of Sciences (CAS) Knowledge Creative Program (Grant No. KSCX2-EW-R-07).


Received: May 30, 2011
Revised: June 21, 2011
Published online: September 8, 2011