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One-pot synthesis of magnetite-loaded dual-mesoporous silica spheres for T₂-weighted magnetic resonance imaging and drug delivery

Xiaofeng Luo, Dechao Niu, Yao Wang, Yungang Zhai, Jianzhuang Chen, Jinlou Gu, Jianlin Shi and Yongsheng Li

The combination of mesoporous silica nanoparticles and superparamagnetic nanocrystals to fabricate multifunctional platforms presents great potentials for simultaneous imaging and drug delivery. In this work, we have successfully developed a simple one-step approach to synthesize magnetite-loaded dual-mesoporous silica spheres consisting of large pores in the core and small pores in the shell (Fe₃O₄@DMSSs) by embedding oil-soluble Fe₃O₄ into the large pores of DMSSs, which were prepared by employing polystyrene-b-poly (acrylic acid) (PS-b-PAA) and cetyltrimethyl ammonium bromide (CTAB) as dual-templates. The loading amounts of magnetite can be easily adjusted by varying the initial concentrations of Fe₃O₄ nanoparticles in the oil phase. The in vitro test indicates that Fe₃O₄@DMSSs possesses excellent T₂-weighted magnetic resonance (MR) imaging performance with a maximum T₂ relaxivity (r₂) of 421.5 mM⁻¹S⁻¹. Furthermore, a high doxorubicin (DOX) loading capacity (65 wt%) was achieved and the obtained DOX-loaded Fe₃O₄@DMSSs (DOX/Fe₃O₄@DMSSs) exhibits pH-sensitive behaviour with accelerated release of DOX in acidic environment. Confocal laser scanning microscopy observation shows that DOX/Fe₃O₄@DMSSs was able to locate in the cytoplasm of MCF-7 cells and release DOX into the nucleus to kill cancer cells. Therefore, it is anticipated that Fe₃O₄@DMSSs can be promising candidates as both T₂-weighted MR contrast agents and drug delivery carriers in further biomedical applications.

Introduction

With the rapid development of nanotechnology, great attention and efforts have been devoted to the design and fabrication of various types of nanomaterials for biomedical applications, such as imaging, diagnosis and therapy. Among these nanomaterials, magnetic nanoparticles, especially for iron oxide nanoparticles, a unique class of functional nanocrystals that plays an important role in research and development of wide application fields such as magnetic fluids, bio-separation and magnetic resonance imaging (MRI), have attracted intensive attention owing to their excellent magnetism, good biocompatibility, tunable sizes and increased contrast enhancement. To date, several methods have been developed for synthesizing superparamagnetic iron oxide nanoparticles, such as co-precipitation, microemulsion, thermal decomposition and hydrothermal treatment. Among these, thermal decomposition has been widely employed to synthesize iron oxide nanoparticles as MRI contrast agents owing to their controllable size, monodispersity and high crystallinity. However, the hydrophobic feature of thus obtained magnetic nanoparticles greatly limits its applications in medicine. In order to improve its dispersion and stability in aqueous systems, various materials such as polyethylene glycol (PEG), dimercaptosuccinic acid (DMSA), amphiphilic polymers and silica have been employed for coating and stabilizing magnetic nanoparticles to extend their applications.

Mesoporous silica nanoparticles (MSNs), as one of the most promising inorganic drug delivery carriers, have been widely investigated due to their unique physicochemical properties including high specific surface area and large pore volume, tunable pore sizes and morphologies, good biocompatibility and easily modified outer/inner surfaces. Recently, the combination of MSNs and iron oxide nanoparticles to prepare magnetic mesoporous silica nanoparticles (M-MSNs) has been widely exploited due to their potentials for simultaneous MRI and drug delivery. Hyeon et al. reported the synthesis of core-shell MSNs by using single Fe₃O₄ nanocrystals as cores (Fe₃O₄@mSiO₂) and demonstrated their good performance in T₂-weighted MRI and drug delivery. J. Zink et al. described the fabrication of multifunctional nanoparticles with several iron oxide nanocrystals encapsulated within MSNs and anticancer drugs stored inside the pores, and the materials exhibited great potential in simultaneous imaging and therapeutic applications. Recently, Shi et al. reported the fabrication of a multifunctional platform for bio-imaging and anticancer drug delivery by integrating a hollow iron oxide nanocapsule with a mesoporous silica shell. Unfortunately, these M-MSNs always have...
relatively low transverse relaxivity \( (r_2 < 300 \text{ mMFe}^{-1} \text{s}^{-1}) \) as single or fewer superparamagnetic iron oxide nanoparticles have been loaded into the mesoporous structure. Moreover, the pore sizes of the reported M-MSNs are relatively small (2-5 nm), which limits the drug loading capability and treatment effect of M-MSNs. To address these issues, herein, we report a simple one-step approach to construct a novel nanocarrier platform for both high transverse relaxivity and efficient drug delivery based on magnetite-loaded dual-mesoporous silica spheres \( (\text{Fe}_3\text{O}_4@\text{DMSSs}) \), which is consist of large pores in the core and small pores in the shell by embedding multiple \( \text{Fe}_3\text{O}_4 \) nanoparticles into the large pores of DMSSs. To exploit its potential biomedical applications, the efficacy in \( T_2 \)-weighted magnetic resonance imaging (MRI), drug storage capacity and potential biomedical applications were investigated.

**Results and discussion**

Fig. 1 shows the representative TEM images of \( \text{Fe}_3\text{O}_4@\text{DMSSs} \) prepared with different loading amounts of \( \text{Fe}_3\text{O}_4 \) nanoparticles (5, 15 and 30 mg) with average diameter of 6 nm (Fig. S1a), denoted as \( \text{Fe}_3\text{O}_4@\text{DMSSs}-5, \text{Fe}_3\text{O}_4@\text{DMSSs}-15 \) and \( \text{Fe}_3\text{O}_4@\text{DMSSs}-30 \), respectively. As shown in Fig. 1a, it is clearly observed that well-defined and core-shell structured dual-mesoporous silica spheres with \( \text{Fe}_3\text{O}_4 \) nanoparticles (dark or black dots in Fig. 1b) encapsulated in the large pores in the core were obtained. On increasing the loading amount of \( \text{Fe}_3\text{O}_4 \) nanoparticles, more magnetic nanoparticles were found incorporated into the large pores (Fig. 1c and d). Further increasing it from 15 to 30 mg, almost all the large pores was filled with magnetic nanoparticles (Fig. 1e and f). In the XRD patterns, all the three \( \text{Fe}_3\text{O}_4@\text{DMSSs} \) present five distinct diffraction peaks in the range of 25-65\(^\circ\) (Fig. S2), which can be assigned to 220, 311, 400, 511 and 440 reflections of the \( \text{Fe}_3\text{O}_4 \) crystal phase with space group of Fd-3m (ICPDS Card Number: 19-0629) (Fig. S1b), confirming the successful encapsulation of \( \text{Fe}_3\text{O}_4 \) nanoparticles in DMSSs. Moreover, the intensities of the diffraction peaks increase with the loading amounts of \( \text{Fe}_3\text{O}_4 \) nanoparticles, suggesting that more magnetite nanoparticles were embedded in the large pores.

![Fig. 1 TEM images of \( \text{Fe}_3\text{O}_4@\text{DMSSs} \).](image)

**Fig. 1** TEM images of \( \text{Fe}_3\text{O}_4@\text{DMSSs} \)-5 (a, b), \( \text{Fe}_3\text{O}_4@\text{DMSSs}-15 \) (c, d), and \( \text{Fe}_3\text{O}_4@\text{DMSSs}-30 \) (e, f).

To investigate the effect of the introduction of \( \text{Fe}_3\text{O}_4 \) on the pore structure of DMSSs, \( \text{N}_2 \) sorption analysis was conducted and the corresponding isotherms and pore size distribution curves are shown in Fig. 2. It is found that all the three samples exhibit type IV isotherms with two major capillary condensation steps at relative pressure of 0.2-0.3 and 0.85-0.95, respectively, implying the dual-mesoporous structure of the samples. Noticeably, the encapsulation of \( \text{Fe}_3\text{O}_4 \) nanoparticles did not change the core-shell dual-mesoporous structure of DMSSs, though the pore diameter distribution of larger pores becomes broader due to the introduction and accumulation of hydrophobic \( \text{Fe}_3\text{O}_4 \) nanoparticles within different large pores. Table 1 presents the synthetic and structural parameters of the samples. It is found that the specific surface area and pore volume are as high as 832 m\(^2\)g\(^{-1}\) and 1.12 cm\(^3\)g\(^{-1}\) for \( \text{Fe}_3\text{O}_4@\text{DMSSs}-5 \), 760 m\(^2\)g\(^{-1}\) and 1.03 cm\(^3\)g\(^{-1}\) for \( \text{Fe}_3\text{O}_4@\text{DMSSs}-15 \) and 842 m\(^2\)g\(^{-1}\) and 0.97 cm\(^3\)g\(^{-1}\) for \( \text{Fe}_3\text{O}_4@\text{DMSSs}-30 \), respectively. The loading amounts of \( \text{Fe}_3\text{O}_4 \) nanoparticles in the DMSSs determined by ICP analysis are about 4.8 wt% for \( \text{Fe}_3\text{O}_4@\text{DMSSs}-5 \), 13.3 wt% for \( \text{Fe}_3\text{O}_4@\text{DMSSs}-15 \) and 24.3 wt% for \( \text{Fe}_3\text{O}_4@\text{DMSSs}-30 \), respectively. In addition, the hydrodynamic diameter testing results by dynamic light scattering (DLS) (Fig. S3) indicate that the particle size increases gradually with the adding amounts of magnetite particles. The mean particle size of the samples is ca. 234, 247 and 276 nm, respectively, with narrow particle distributions, demonstrating the good monodispersity of \( \text{Fe}_3\text{O}_4@\text{DMSSs} \) in aqueous solution. These verify that monodisperse, dual mesoporous silica nanospheres loaded with \( \text{Fe}_3\text{O}_4 \) nanoparticles could be facilely fabricated with one-pot synthesis.
To explore the drug delivery performance of Fe₃O₄@DMSSs, doxorubicin (DOX), a chemotherapeutic drug was chosen as a model and loaded into the pores of Fe₃O₄@DMSSs-5 via the strong electrostatic interaction between the positively charged DOX and negatively charged pore channels. The DOX loading process can be monitored by UV-vis absorbance spectrometry. As shown in Fig. S5, the intensity of the absorbance peak at 480 nm, which is the characteristic wavelength of DOX, decreases with the loading process, demonstrating the successful loading of DOX into Fe₃O₄@DMSSs-5. The DOX loading content was measured to be 65 wt%, which is much higher than that of the reported magnetic mesoporous silica nanoparticles. On the other hand, the significant decrease in both specific surface area and pore volume of DOX/Fe₃O₄@DMSSs-5 (44 m²·g⁻¹ and 0.09 cm³·g⁻¹) further indicates that most of the pores has been occupied by the adsorbed DOX drug molecules.

It is of great importance to investigate the drug release feature of Fe₃O₄@DMSSs as a practical drug delivery system for cancer chemotherapy. As a result, the in vitro DOX release properties of DOX/Fe₃O₄@DMSSs-5 were examined in PBS solution at various pH values (Fig. 4). It is showed that the DOX release behaviour of DOX/Fe₃O₄@DMSSs-5 is pH-dependent. In details, in a pH=7.4 solution, only about 8.5 % of DOX was released within 18 h, which is vitally important to maintain the drugs less cytotoxic to the normal cells. By increasing the acidity of the solution to a pH value of 6.4, the release amount reached 18% within 24 h, and in a more acidic solution of pH 5.4, a sustained release followed by a fast release within the first 10 h was obtained, and about 40% of DOX was released from the nanocarriers in 72 h. This pH-responsive drug release behavior is attributed to the following two factors: (1) at lower pH values, the protonation of amine groups on DOX molecules (pKa=8.2) become stronger, which could increase the hydrophilicity and solubility of DOX molecules; (2) the decreased pH would weaken the electrostatic interaction between negatively charged Fe₃O₄@DMSSs and positively charged DOX molecules, resulting in the faster release rate, which was similar with other reported literatures. Additionally, the pH dependent release character of the carriers may benefit the DOX release in relative acidic tumour microenvironment. Meanwhile, it is desirable that most DOX encapsulated in the pore channels would not leach out during in vivo circulation in the blood with a pH value of 7.4, and enable large amount of intracellular drug released once the nanoparticles are internalized inside the tumour cells by endocytosis as endosome/lysosome has a low pH value.

<table>
<thead>
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<th>Sample</th>
<th>Fe₃O₄ amount (mg)</th>
<th>loading amount (wt %)</th>
<th>dₕ(Å)</th>
<th>dₜ(Å)</th>
<th>S/m²</th>
<th>η/min·g⁻¹</th>
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<td>Fe₃O₄@DMSSs-5</td>
<td>5</td>
<td>4.8</td>
<td>2.2</td>
<td>17/21.8</td>
<td>832</td>
<td>1.12</td>
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<tr>
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<td>15</td>
<td>13.3</td>
<td>2.2</td>
<td>17/22</td>
<td>760</td>
<td>1.03</td>
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<tr>
<td>Fe₃O₄@DMSSs-30</td>
<td>30</td>
<td>24.3</td>
<td>2.2</td>
<td>17</td>
<td>842</td>
<td>0.97</td>
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To evaluate the T₂-weighted MR imaging capability of Fe₃O₄@DMSSs, the transverse relaxation was measured by using a clinical 3.0 T MRI scanner and the transverse (r₂ value) relaxivity was calculated through the curve fitting of 1/ T₂ relaxation time versus the Fe concentration (Fig. 3). The result shows that the r₂ value increases with the increasing of Fe₃O₄ loading amount. It is calculated to be 370.2 mM mMFe⁻¹·S⁻¹ for Fe₃O₄@DMSSs-5, 385.8 mM MFe⁻¹·S⁻¹ for Fe₃O₄@DMSSs-15 and 421.5 mM MFe⁻¹·S⁻¹ for Fe₃O₄@DMSSs-30, respectively. The magnetic properties of Fe₃O₄@DMSSs-5, Fe₃O₄@DMSSs-15 and Fe₃O₄@DMSSs-30 were measured by using vibrating sample magnetometer (VSM). As shown in Fig. S4, the room-temperature magnetization curves show no hysteresis loop, demonstrating the superparamagnetic feature of all the three samples. In addition, the saturation magnetization values of these samples are calculated to be 1.5 emu per gram of Fe₃O₄@DMSSs-5, 3.6 emu per gram of Fe₃O₄@DMSSs-15 and 5.5 emu per gram of Fe₃O₄@DMSSs-30, respectively.

The magnetic properties of Fe₃O₄@DMSSs-5, Fe₃O₄@DMSSs-15 and Fe₃O₄@DMSSs-30 were measured by using vibrating sample magnetometer (VSM) (Fig. 3). The result shows that the r₂ value increases with the increasing of Fe₃O₄ loading amount. It is calculated to be 370.2 mM mMFe⁻¹·S⁻¹ for Fe₃O₄@DMSSs-5, 385.8 mM MFe⁻¹·S⁻¹ for Fe₃O₄@DMSSs-15 and 421.5 mM MFe⁻¹·S⁻¹ for Fe₃O₄@DMSSs-30, respectively. The magnetic properties of Fe₃O₄@DMSSs as a novel kind of T₂-weighted MR contrast agent. Noticeably, the r₂ values of Fe₃O₄@DMSSs are much higher than that of commercial iron oxide-based contrast agent (Feridex, r₂ = 108 mMFe⁻¹·S⁻¹) and most of the iron oxide-based mesoporous nanoparticles. This is probably attributed to the dual-mesoporous structure, which is feasible for water molecules to contact magnetite nanoparticles, resulting in shortened transverse relaxation period and consequently intensified MR imaging performance. Besides, the synergetic effect between the multiple magnetite nanoparticles is also responsible for this.
In order to investigate the cellular uptake of Fe₃O₄@DMSSs, fluorescein isothiocyanate (FITC) was chosen as a marking dye to graft onto the surface of the nanoparticles (denoted as FITC-Fe₃O₄@DMSSs) via silane conjugation chemistry. The obtained FITC-Fe₃O₄@DMSSs were incubated with MCF-7 cells for 4 h at 37°C in the culture medium, and analyzed by confocal laser scanning microscopy (CLSM). As shown in Fig. 5a, it is observed that FITC-Fe₃O₄@DMSSs were internalized by the MCF-7 cells and localized in the cytoplasm within 4 h of incubation. The internalization is clearly evident since the MCF-7 cell nucleus was stained with DAPI blue dye, suggesting the cellular uptake instead of adherence to the surface of particles. In addition, the intracellular distribution of DOX/Fe₃O₄@DMSSs in MCF-7 cells was estimated. As shown in Fig. 5b, strong fluorescence was emitted from the cells treated with DOX/Fe₃O₄@DMSSs solution, particularly the nuclear regions after 4 h of incubation in MCF-7 cells, indicating that DOX/Fe₃O₄@DMSSs was efficiently internalized in MCF-7 cells by nonspecific endocytosis. Furthermore, Fe₃O₄@DMSSs could be transported into the nuclei while free DOX released from DOX/Fe₃O₄@DMSSs was able to enter the nuclei by passive diffusion, which was demonstrated by the presence of strong red fluorescence emitted from nuclei of MCF-7 cells treated with DOX/Fe₃O₄@DMSSs. These may be attributed to the fast release of DOX in the low pH region in the cells (e.g., the pH values of endosomes and lysosomes are ca. 5.0-5.5) [17], which is consistent with the in vitro release profiles of DOX/Fe₃O₄@DMSSs (Fig. 4). From the above observations, it is concluded that DOX/Fe₃O₄@DMSSs could penetrate into the living cells and thus the loaded DOX could be released from the nanocarriers.

![Fig. 5 CLSM images of MCF-7 cells incubated with FITC-Fe₃O₄@DMSSs (a) and DOX/Fe₃O₄@DMSSs (b) for 4 h.](image)

To further verify whether the released DOX was still pharmacologically active, in vitro cytotoxicity tests against MCF-7 cells were investigated. Cell viabilities against DOX/Fe₃O₄@DMSSs and free DOX at different concentrations are shown in Fig. 6. The results reveal that the cytotoxic efficacy of the DOX/Fe₃O₄@DMSSs was comparable to free DOX after incubation with MCF-7 cells for 24 h. Moreover, more than half of tumor cells were effectively killed when incubated with DOX/Fe₃O₄@DMSSs for 24 h, indicating that DOX delivered by Fe₃O₄@DMSSs entered the MCF-7 cells and retained its pharmacological activity. In contrast, the DOX-free Fe₃O₄@DMSSs showed very low cytotoxicity against MCF-7 cells as well as representative normal cells (L02 normal human liver cells) (Fig. S6). Consequently, Fe₃O₄@DMSSs has been proved to be one of promising nanocarrier candidates in drug loading and delivery in further cancer chemotherapy.

![Fig. 6 In vitro cytotoxicity of free DOX and DOX/Fe₃O₄@DMSSs against MCF-7 cells in 24 h of incubation.](image)

**Conclusion**

In summary, a one-step route has been successfully developed to synthesize magnetite-loaded dual-mesoporous silica spheres (Fe₃O₄@DMSSs) consisting of large pores in the core and small pores in the shell for T₂-weighted magnetic resonance imaging and drug delivery. The obtained Fe₃O₄@DMSSs displays a well-fine core-shell morphology and good monodispersion. Moreover, Fe₃O₄@DMSSs presents excellent T₂-weighted MR imaging effect with a high T₂-relaxivity (r₂ > 350 mM⁻¹ s⁻¹). In addition, Fe₃O₄@DMSSs shows high loading capacity (65 wt%) for doxorubicin due to its unique dual-mesoporous structure with high specific surface area and pore volume. More importantly, the cytotoxicity of DOX-loaded Fe₃O₄@DMSSs against MCF-7 cells is comparable to free DOX at relatively low drug concentrations owing to the intracellular release of drugs from DOX-loaded Fe₃O₄@DMSSs in cells. These unique properties endow them with great application potentials as anticancer drug carriers for the simultaneous imaging diagnosis and chemotherapy applications in future.

**Experimental section**

**Chemicals and Materials**

Cetyl trimethyl ammonium bromide (CTAB, ≥ 99%), ammonia solution (25-28%) and tetraethyl orthosilicate (TEOS, AR) were purchased from Shanghai Lingfeng Chemical Reagent Co. LTD. Tetrahydrofuran (THF, AR) and ethanol (AR) were purchased from Sinopharm Chemical Reagent Co. LTD (Shanghai, China). The pure water with a resistivity of 18.2 MΩ cm was used in all of experiments. All of reagents were used without further purification.
Synthesis of PS100-b-PAA16 and magnetite nanoparticles

 Amphiphilic block copolymer, polystyrene100-b-poly (acrylic acid)16 (PS100-b-PAA16), was synthesized via sequential atomic transfer radical polymerization (ATRP) as previously reported. Monodispersed 6 nm sized and hydrophobic Fe3O4 nanoparticles were prepared following the thermal decomposition method reported by Sun et al.11

Synthesis of magnetite-loaded dual-mesoporous silica spheres (Fe3O4@DMSSs)

Fe3O4@DMSSs was prepared according to the previous method reported by our group. In a typical synthesis, 0.05 g of PS100-b-PAA16 and 5 mg of Fe3O4 were first dissolved in 10 mL of THF. Then the above oil solution was poured into a mixture solution containing 40 mL of H2O, 0.065 g of CTAB and 1.5 mL of ammonia. After that, 80 mL of ethanol was added into the mixture oil-water solution. After stirring for 2 h, 0.3 g of TEOS dissolved in 5 mL of ethanol was added into the above solution with continuous stirring in 0.5 h. After stirring for 18 h at room temperature, the as-synthesized sample was collected by centrifugation (1000 t/min, 10 min) and washed several times with water and ethanol. Finally, the product was obtained by dried in an oven and calcined at 550°C for 6 h for surfactants removal.

In vitro MR imaging

The in vitro MR imaging experiment was performed on a 3.0 T clinical MRI instrument (GE Signa HDx 3.0 T). For the T2-weighted fast-recovery fast spin-echo (FR-FSE) sequence, the following parameters were used: TR (repetition time) = 2000 ms, TE (echo time) = 107.1 ms, Field of view (FOV) = 14 ms, slice thickness = 2.0 mm, echo length = 16, matrix = 256 × 192, number of acquisitions= 4. For T2 relaxation measurement, firstly, the Fe concentration of the Fe3O4@DMSSs in water was determined by inductively coupled plasma atomic emission spectrometry (ICP-AES) after dissolving the samples in a mixture solution of HNO3/HClO4 at 150 °C. Then, solutions of samples containing different Fe concentrations were prepared in pure water. The T2 relaxation time was performed with the following parameters: TR = 4000 ms, TE= 13, 26, 39, 52 ms. Relaxivity values of r2 were calculated through the curve fitting of 1/T2 relaxation time (s-1) versus the Fe concentration (mM).

DOX storage and release

Typically, 15 mg of DOX was completely dissolved in 10 mL of phosphate buffer solution (PBS). Then, the 20 mg of Fe3O4@DMSSs-5 was added into the DOX-PBS solution stirred for 24 h in dark at room temperature. The products were washed quickly several times with PBS to remove the physically adsorbed DOX residue on the surface and dried in a vacuum oven for 24 h. The concentration of the drug retained in the solution was determined by UV-vis spectrometer at 480 nm. The loading amount of the drug was calculated according to margin of the initial and the residual drug. In vitro drug release experiments were carried out in PBS at different pH values (5.4, 6.4 and 7.4). The DOX-loaded Fe3O4@DMSSs-5 (5 mg) were suspended in 3 mL PBS in the dialysis membrane bag (molecular weight cut-off 3,500 Da) and the bag was immersed in 27 mL PBS and shaken at a speed of 100 rpm at 37°C. At predetermined time intervals, 3.0 mL of the release buffer was removed from the tube, and then 3.0 mL of the fresh buffer was added to make the loss of solution.

The collected buffer samples were examined by a UV-vis spectrometer to determine the concentration of the DOX.

In vitro cellular uptake

To observe cellular uptake of Fe3O4@DMSSs, MCF-7 cells were cultured for 12 h at 37°C in Dulbecco’s Modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum (FBS) in a 35 mm confocal dish and incubated for 24 h at 37°C. After that, FITC-Fe3O4@DMSSs and DOX/Fe3O4@DMSSs were added into the dishes at a concentration of 100 μg/mL, respectively. After incubation for 4 h, cells were washed for several times with PBS, fixed with 4% paraformaldehyde, and stained with 4’, 6-diamidino-2-phenylindole (DAPI) for 5 min followed by washing with PBS. The fluorescence images were acquired by confocal laser scanning microscopy (CLSM).

In vitro cytotoxicity

For the cytotoxicity of free DOX, parent Fe3O4@DMSSs and DOX/Fe3O4@DMSSs against MCF-7 cells and the biocompatibility of parent Fe3O4@DMSSs with L02 cells (normal human liver cells), cells were cultured in DMEM containing 10% FBS. Cells were seeded in 96-well plates at a density of 104 cells per well and cultured in 5% CO2 at 37°C for 24 h. Then, free DOX, parent Fe3O4@DMSSs and DOX/Fe3O4@DMSSs were added to the culture medium, and the cells were incubated in 5% CO2 at 37°C for 24 h. The concentrations of DOX were set at 0, 1.25, 2.5, 5, 10 and 20 μg/mL, and the concentrations of parent Fe3O4@DMSSs were 0, 10, 25, 50 and 200 μg/mL. Cell viability was determined using the standard 3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide (MTT) assay. The statistical analysis of the experiments data utilized the Student’s t-test. Each data point is represented as mean ± standard deviation (SD) of three independent experiments.

Characterization

X-ray diffraction (XRD) patterns were obtained on Rigaku D/Max-2200PC using Cu Kα radiation (40 kV, 40 mA). Transmission electron microscopy (TEM) images were obtained on a JEM-2100F electron microscope operating at 200 kV. The samples were suspended in ethanol and then transferred onto a copper mesh coated with an amorphous carbon film for TEM measurements. Nitrogen adsorption-desorption isotherms at 77 K were measured on a Quantachrome NOVA 4200c. The specific surface area and the pore size distribution were calculated by using the Brunauer-Emmett-Teller (BET) and the Barrette-Joyner-Halenda (BJH) methods, respectively. Dynamic light scattering (DLS) measurements were performed using a Zeta potential/particle Sizer Nicomp TM 380 ZLS (PSS Nicomp particle size system, U.S.A.). UV-vis spectra were recorded on a Shimadzu UV-2550 spectrometer.

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Notes and references

† Low dimensional Materials Chemistry Laboratory, School of Materials Science and Engineering, East China University of Science and Technology, Shanghai, 200237, China. E-mail: ysl@mail.ecust.edu.cn; dcniu@mail.ecust.edu.cn; Tel: +86-21-64250740.

‡ State Key Laboratory of High Performance Ceramics and Superfine Microstructures, Shanghai Institute of Ceramics, Chinese Academy of Sciences, 1295 Dingxi Road, Shanghai, 200050, China.

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