Nanoengineering of Core–Shell Magnetic Mesoporous Microspheres with Tunable Surface Roughness

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ABSTRACT: Functional core–shell mesoporous microspheres with integrated functions, controlled structure, and surface properties and morphologies have received increasing attention due to their excellent physicochemical properties. Herein, core–shell magnetic mesoporous materials with cauliflower-like morphology and tunable surface roughness have been synthesized through a kinetics-controlled interface co-assembly and deposition of mesostructured nanocomposites on Fe3O4@RF microspheres (RF refers to resorcinol formaldehyde resin). The obtained microspheres, synthesized via this interface nanoengineering method, possess well-defined sandwich structure with a tunable rough morphology, uniform size (∼560–1000 nm), perpendicularly aligned mesopores (∼5.7 nm) in the outer shell, RF-protected magnetic responsive core, high surface area up to 382 m2/g, and large pore volume of 0.66 cm3/g. As a result of the unique surface features and magnetic properties, these microspheres exhibit excellent performance in stabilizing and oxygen-free manipulating aqueous solutions in petroleum ether by a magnetic field. They also exhibit superior cell uptake properties compared with traditional smooth core–shell magnetic mesoporous silica microspheres, opening up the possible applications in fast drug delivery in cancer therapy.

INTRODUCTION

In the past decades, core–shell microspheres with different discrete functions integrated into one structure have attracted increasing research interest due to their promising potential applications in advanced biomedicine, renewable energy, applied catalysis, and environmental cleanup.†–14 To date, great progress has been made in designing core–shell nanostructures with diverse shell compositions, tunable thickness and different structures, and various functional cores.14–21 Besides, various surface topologies of core–shell structures endow the microspheres intriguing and unique physical and chemical properties, thus achieving distinctive applications.22–25 A typical Mother Nature inspired masterpiece is mimicking the surface roughness topology of lotus leaves for construction of superhydrophobic and self-cleaning films.24–27 Due to the intriguing rough morphology, microspheres with convex surface morphology, similar to the raspberry morphology, were widely investigated for construction of super-hydrophobic and self-cleaning surface.26,29 Moreover, particles with analogous pollen and virus structure were recently demonstrated to exhibit enhanced adhesion toward “hairy” objects and superior cell uptake properties.30–32 Until now, researchers have achieved the synthesis of raspberry-like microspheres with rough surface via attaching small particles like silica nanogels on the core of organic polymer microspheres (e.g., polystyrene and poly(methyl methacrylate)) through electrostatic adsorption force or hydrogen-bond interactions by Pickering emulsion polymerization33–39 or layer-by-layer (LBL) assembly methods.30–42 The obtained raspberry-like composite microspheres were proved to have a unique superhydrophobic surface similar to the surface property of lotus leaves.

However, these previously reported raspberry-like spheres are solid and only possess rough surfaces. Considering the special adhesion behavior of these rough surfaces, it is highly

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interesting to impart them with a porous structure, which can provide an add-on function of high surface areas and large pore volume for accommodation of guest objects (e.g., drug) to be controlled delivered and released through pore channels after attachment to sites of interest. Recently, Song et al. reported hollow mesoporous silica microspheres with a rough surface through an one-pot synthesis of organic polymer—silica composite microspheres and subsequent calcination to remove the polymer component. Despite their hydrophilic composition, these hollow mesoporous silica microspheres exhibit a hydrophobic surface property, enabling good affinity and adhesion toward Escherichia coli and sustained release of preloaded lysozymes for long-term bacterial inhibition. From the viewpoint of a practical application, it is desired to design porous microspheres with rough surfaces, high porosity, and integrated functionalities (e.g., photo-, magnetic, and electro properties) to improve their comprehensive performances and broaden their applications. In addition, to simplify the manipulation-like separation and recycling and control the release rate of guest cargos, core—shell structures with a magnetic core are ideal candidates due to their fast magnetic responsive and magnetic-thermal properties. Until now, there is no report on the designed synthesis of core—shell mesoporous microspheres with a rough and adjustable surface topology and open and ordered mesopore channels as the shell and magnetic responsive core.

Herein, we report a rational nanoengineering of a novel kind of cauliflower-like core—shell microspheres with tunable surface roughness, magnetic core, and mesoporous silica shell. Such magnetic mesoporous silica microspheres have cauliflower-like morphology (denoted as CL-MMS microspheres) and were synthesized through a well-controlled interface engineering method which involves the successive deposition of resorcinol-formaldehyde (RF) on magnetite particles via an interface polymerization and mesoporous silica layer via an organic— inorganic assembly process. Core—shell microspheres possess uniform and adjustable diameters ranging from 560 nm to 1000 μm, superparamagnetic property with a high magnetization (35.1 emu/g), mesoporous silica shell with tunable thickness of 30–250 nm, and perpendicularly aligned mesopore channels (5.7 nm in diameter). Moreover, the surface roughness morphology and hydrophobicity of single particle can be well tuned by controlling the kinetics process of interfacial nucleation and growth of mesostructure CTAB-silica composites. The designed CL-MMS microspheres show an interesting capability in stabilizing and oxygen-free manipulation of aqueous solutions in hydrophobic solvents. Moreover, the rough outer surface morphology endows the microspheres enhanced hydrophobic outer surface properties and superior cell uptake efficiency toward P02 cells, opening up the application possibility of drug delivery in cancer therapy.

## EXPERIMENTAL SECTION

### Chemicals.
FeCl₃·6H₂O, trisodium citrate, sodium acetate, tetraethyl orthosilicate (TEOS), resorcinol, formaldehyde, ethanol, ethylene glycol, n-hexane, and concentrated ammonia solution (28 wt %) are of analytical grade (Shanghai Chemical Corp.). Cetyltrimethylammonium bromide (CTAB), fluorescein isothiocyanate (FITC), and aminopropyltriethoxysilane (APTES) were supplied by Sigma-Aldrich. The murine pancreatic ade-nocarcinoma cell line Pan02 was purchased from the Frederick National Laboratory for Cancer Research (Maryland, USA). Roswell Park Memorial Institute (RPMI) 1640 and fetal bovine serum (FBS) were obtained from Gibco (California, USA). All other chemicals were used as received. Deionized water was used for all experiments.

### Synthesis of Fe₃O₄@RF Microspheres.
Magnetic Fe₃O₄ particles with a mean diameter of ~300 nm were synthesized via a solvothermal reaction according to a previous report. 100 mg of Fe₃O₄ particles was dispersed in a mixture solution of ethanol (20 mL) and deionized water (10 mL) by ultrasonication. Into the resultant dispersion, concentrated ammonia solution (0.50 g, 28 wt %), resorcinol (0.10 g, 0.09 mM), and formaldehyde (0.10 g, 37 wt %) were added consecutively. The mixed dispersion was mechanically stirred for 10 h at 30 °C for the interface polymerization of resorcinol and formaldehyde on Fe₃O₄ particles by virtue of the iron-phenol complexation, and subsequently the temperature was increased to 100 °C to allow a full cross-linking of RF resin on Fe₃O₄ particles. The obtained core—shell Fe₃O₄@RF microspheres were collected by a magnet and washed with deionized water and ethanol three times, respectively.

### Synthesis of CL-MMS Microspheres with Rough Surface.
In a typical synthesis, 0.20 g of Fe₃O₄@RF microspheres were dispersed in a mixed solution consisting CTAB (0.50 g, 1.3 mmol), deionized water (80 mL), and concentrated ammonia solution (0.80 mL, 28 wt %) with ultrasonication. Into the water solution, 5 mL of n-hexane solution containing TEOS (2.0 mL) was added dropwise for 30 min. The reaction was kept stirring for 12 h at 20 °C, and then the product was collected with a magnet and washed with ethanol and water three times, respectively. Finally, the microspheres were redispersed in 60 mL of ethanol and refluxed at 70 °C overnight to remove CTAB templates. The extraction was repeated 3 times and then thoroughly washed with ethanol. After vacuum drying, cauliflower-like core—shell magnetic mesoporous silica microspheres (denoted as CL-MMS microspheres) were obtained. To control the hydrolysis and condensation of TEOS and the co-assembly of CTAB and silica species during the interface co-assembly, similar syntheses were carried out according to the above procedure by only changing the amount of ammonia from 0.50 to 2.0 mL. Notably, CL-MMS microspheres can only maintain their structure at temperatures below 400 °C. Thermal treatment at higher temperatures can burn out RF resin and destroy their structure.

### Synthesis of Core—Shell Magnetic Mesoporous Silica (MMS) Microspheres with Smooth Surface.
The synthesis procedure is similar to that for CL-MMS microspheres, except that the reaction temperature was 35 °C instead of 20 °C, and meanwhile the amount of n-hexane was 20 mL instead of 5.0 mL.

### Measuring the Contact Angle of a Single Particle.
The particle contact angle of CL-MMSs with different roughness was measured through a gel trapping technique (GTT) which was first developed by Paunov in 2003. First, CL-MMS microspheres were injected into the static and stable oil—water (decane-water with 2% gellan) interface. As the microspheres spread stably at oil—water interface, the two phase system was cooled to set the gel. The sublayer water with 2% gellan became a gelled water phase. Thus, the particle monolayer remains embedded on the gel surface, and the particle positions with respect to the liquid interface remain fixed. Then, the top decane was removed and replaced with a poly(dimethylsiloxane) (PDMS) silicone elastomer. After curing the PDMS, the particle monolayer was peeled off the aqueous gel and imaged on the PDMS surface. HRSEM images from the GTT were observed using JEOL 7800 field operated at 15 kV. A thin carbon layer of about 10 nm was deposited over the particle monolayer on the PDMS surface prior to SEM imaging. The particle contact angle measured through the PDMS phase is complementary (with respect to 180 °C) to the particle contact angle in the original system (oil—water interface). To reduce the measurement error, the contact angles of multiple (n represents the number of microspheres measured) microspheres were measured and averaged for each sample.

### Cell Uptake Assay.
The as-synthesized CL-MMS microspheres were labeled with fluorescein isothiocyanate according to a previously reported method to facilitate the observation of cell uptake of CL-MMS microspheres by confocal microscopy. Typically, FITC-APTES molecules were obtained by the reaction of APTES (10 mg) with...
FITC (5 mg) in 20 mL of absolute ethanol at 25 °C with stirring in the dark for 8 h. Into the resultant solution, an ethanol solution (10 mL) containing 20 mg of CL-MMS microspheres was injected, and the solution was further stirred for 12 h in the dark. The magnetic microspheres were collected by centrifugation, washed with ethanol three times, and finally vacuum-dried at 25 °C in the dark for 12 h. Thus, the FITC-labeled microspheres (denoted as FITC-CL-MMS microspheres) were obtained.

Pan02 cells (~3 × 10^5) were seeded in a 6-well plate containing a sterilized coverslip. After overnight incubation, the cells adhere to the coverslips, and the culture medium was replaced with 2 mL of complete medium containing 15 μL of CL-MMS microspheres (2.0 wt %) or MMS microspheres with a smooth surface (2.0 wt %). After incubation for another 0.5, 1, 2, 4 h, respectively, the cells were washed with PBS three times and fixed in 100% acetone for 5 min. Then the cells were observed under CLSM (Leica-SP8). The untreated cells were set as controls.

Measurements and Characterization. Transmission electron microscopy (TEM) experiments were conducted on a JEOL 2011 microscope (Japan) operated at 200 kV. Field-emission scanning electron microscopy (FESEM) images were collected on Hitachi microscope (Japan) operated at 200 kV. Field-emission scanning microscopy (TEM) experiments were conducted on a JEOL 2011 microscope (Japan). Before measurements, the samples were degassed in a vacuum at 100 °C for 10 h. The Brunauer–Emmett–Teller (BET) method was utilized to calculate the specific surface areas using adsorption data in a relative pressure range of 0.005–0.25. By using the Barrett–Joyner–Halenda (BJH) model, the pore volumes and pore size distributions were derived from the adsorption branches of isotherms, and the total pore volumes (Vₚ) were estimated from the adsorbed amount at a relative pressure P/P₀ of 0.995.

■ RESULTS AND DISCUSSION

The overall synthesis procedure of CL-MMS microspheres is depicted in Scheme 1. First, water dispersible Fe₃O₄ particles were obtained via a well-established solvothermal method and coated by a protective shell of RF resin through an interfacial polymerization of resorcinol and formaldehyde in an alkaline solution by virtue of the complexation between phenol groups and Fe₃O₄ particles. Second, a shell of rough CTAB/SiO₂ mesostructured nanocomposite was further deposited on the surface of Fe₃O₄@RF microspheres through an interface deposition and organic–inorganic co-assembly process using TEOS as a silica precursor and CTAB as a structure-directing agent (i.e., the template molecule). Third, the CTAB template was removed by ethanol extraction and core–shell cauliflower-like magnetic mesoporous silica microspheres were obtained.

The as-synthesized Fe₃O₄ particles have a nearly spherical morphology and uniform diameter of about 300 nm, and they consist of magnetite nanocrystals (~7 nm) (Figure S1ab). Such a nanocluster structure of magnetite particle is favorable for a fast magnetic separation process and meanwhile ensures a superparamagnetic property at room temperature. A shell of RF resin was coated on the magnetic particles through the polymerization of resorcinol and formaldehyde at 35 °C in a basic solution due to the strong complexation between iron ions and phenolic hydroxyl groups of resorcinol molecules. SEM observations show that the resultant Fe₃O₄@RF microspheres have a uniform size of about 500 nm and regularly spherical morphology (Figure S1c). TEM images indicate a distinctive core–shell structure with a shell RF thickness of ~100 nm (Figure S1d). Here, the RF obtained via polycondensation at room temperature has a unique hydrophilic/hydrophobic property. Its hydrophobic framework makes Fe₃O₄@RF microspheres show affinity to organic solvents, while the phenol hydroxyl groups in Fe₃O₄@RF microspheres’ surface endow a water dispersibility which is favorable for further deposition and coating process in aqueous solutions.

A shell of mesostructural CTAB/SiO₂ nanocomposites was deposited on the surface of Fe₃O₄@RF microspheres through the co-assembly of CTAB and silicate oligomers from the interface hydrolysis of TEOS in the presence of Fe₃O₄@RF microsphere seeds. The subsequent treatment with refluxing ethanol can remove CTAB templates from the CTAB-silica composite due to the excellent solubility of CTAB in ethanol, yielding a mesoporous silica shell (Figure 1). The obtained CL-

Scheme 1. Synthesis Procedure for the CL-MMS Microspheres via Stepwise Surface Nanoengineering

Figure 1. SEM (a, b) and TEM (c, d) images of CL-MMS microspheres. The inset in panels a and b shows the photograph of a cauliflower and enlarged FESEM image of CL-MMS microsphere, respectively. MMS exhibit a uniform diameter of about 680 nm with a rough surface morphology similar to a cauliflower (Figure 1a and the inset). The enlarged FESEM image (Figure 1b) of single microsphere clearly shows the convex structure with open and closely arranged uniform mesopores of ~6.0 nm (Figure 1b, inset). TEM observations (Figure 1c) reveal a well-dispersed core–shell sandwich structure with a flower-like outer shell due to the two-dimensional projection. All of these microspheres show similar structure and morphology under TEM, implying a uniform cauliflower-like structure. The high-

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resolution TEM image (Figure 1d) clearly shows radially aligned mesopore channels in the outer shell, and the mesoporous shell is about 90 nm in thickness. SEM images with different magnifications (Figure S2) further confirmed the uniformity of CL-MMS microspheres in terms of their size and morphology. To further observe the whole structure of the CL-MMS microspheres, the electron tomography (ET) technique was performed (Movie S1, Supporting Information). For a selected CL-MMS microsphere, a series of tilted TEM images ranging from $-60^\circ$ to $+60^\circ$ at an interval of $1^\circ$ were digitally acquired, and the movie clearly indicates that the embossed structure can be visible at different tilting angles with radially aligned mesoporous channels in the outer shell of the composite microsphere. The cross-sectional TEM specimen was further tilted ranging from $-60^\circ$ to $+60^\circ$ at an interval of $1^\circ$ (Movie S2), which clearly shows convex shell morphology with highly ordered mesopore channels.

A nitrogen adsorption–desorption isotherm (Figure 2a) of CL-MMS microspheres shows a typical type IV curve with a sharp capillary condensation step at a relative pressure around 0.65–0.75, indicating cylindrical pores with large and uniform mesopores. The pore size distribution curve derived from the adsorption branch indicates that CL-MMS microspheres have a mean pore size of 5.7 nm (Figure 2b), much larger than that (usually ~2.2 nm) of traditional mesoporous silica obtained by simply using CTAB as the template. This is mainly due to the swelling effect of n-hexane on the CTAB-silica composite micelles, which can significantly expand the hydrophobic volume of the micelles and result in larger mesopores. The BET surface area and total pore volume were calculated to be 382 m$^2$/g and 0.66 cm$^3$/g, respectively. The wide-angle XRD patterns (Figure S3) show the typical characteristic diffraction peaks assigned to the magnetite phase, indicating that the magnetite component in the composite is well retained in the whole synthesis process. Magnetic property characterization (Figure S4) using a superconducting quantum interference device at 300 K indicates that Fe$_3$O$_4$ and CL-MMS microspheres have high magnetization saturation values of 71.2 and 35.1 emu/g, respectively, which favor a fast separation process for their practical applications. Moreover, no remanence was detected, reflecting a superparamagnetic property due to the presence of nanosized magnetite in the magnetic core of CL-MMS microspheres. Therefore, by using an applied magnetic field, the magnetic microspheres in their water dispersion can be easily manipulated; meanwhile, the aggregated magnetic microspheres can be quickly redispersed with a gentle hand-shaking or sonication after removal of the magnetic field.

More interestingly, it is found that the surface roughness can be tuned by changing the amount of ammonia-water in the synthesis system. For convenience, the microsphere synthesized using different addition amounts of ammonia were denoted as CL-MMS-x ($x$ represents the volume percentage of the concentrated ammonia). With a small fraction of ammonia (0.6 vol %), a thin shell made of uniform mesoporous silica nanospheres (70 nm in diameter) was observed on the surface of Fe$_3$O$_4$@RF microspheres (Figure 3a), and the obtained microspheres exhibit a mean diameter of 560 nm. TEM image shows that CL-MMS-0.6 microspheres clearly have small bump-like mesoporous silica nanospheres adhering to each other in the outer shell (Figure 3b). A high-magnification TEM image (Figure 3c) further confirmed the sandwich-like structure of the microspheres with perpendicularly aligned mesopores in the outer shell. With increasing the dosage of ammonia-water to 1.0 vol %, the obtained microspheres (CL-MMS-1.0) show a nearly regular cauliflower-like structure with open and perpendicular mesopore channels formed on the surface of Fe$_3$O$_4$@RF cores (Figure 3d–f). As the addition of ammonia-water increases to 1.9 vol %, it can be seen that a thick and continuous layer of interconnected granular particles unit with a mean diameter of 300 nm was deposited on the surface of Fe$_3$O$_4$@RF microspheres (Figure 3g). TEM images show that CL-MMS-1.9 microspheres possess clear core–shell–shell structure with radial mesopores in the outer shell (Figure 3h,i). As the amount of ammonia-water reaches 2.5 vol %, the Fe$_3$O$_4$@RF core was coated by a thick layer of polyhedron-
shaped mesoporous silica, leading to CL-MMS-2.5 with a larger diameter of ∼1000 nm (Figure 3j). The edges of the polyhedron shell can be clearly observed, and the ordered mesopore structure is visible in the outer shell. Further increasing the amount of concentrated ammonia-water only led to large aggregated core–shell particles due to the fast hydrolysis and condensation of TEOS on the surface of Fe₃O₄@RF microspheres. To investigate the hydrophobicity of these microspheres with different roughness, core–shell–shell Fe₃O₄@RF@mSiO₂ microspheres (MMS) with a relatively smooth surface were synthesized through a similar method except that the reaction temperature was increased from 20 to 35 °C, and meanwhile the amount of n-hexane was increased from 5 to 20 mL. The obtained MMS microspheres have a mean diameter of about 680 nm with much smoother spherical morphology and distinct core–shell–shell structure (Figure S5), and they have the same perpendicular mesopores (∼6.0 nm) in the outer silica shell, similar to that of CL-MMS microspheres. The hydrophobicity was characterized by dispersing CL-MMS-1 and MMS microspheres with the same diameter (∼680 nm) into diethyl ether-water two phase systems under sonication for 10 s. As shown in Figure 4e, CL-MMS microspheres were found to thermodynamically remain at the diethyl ether–water interface, while MMS microspheres tend to be dispersed completely in the water phase. It implies that CL-MMS microspheres exhibit relative affinity to the hydrophobic layer due to their highly rough surface with enclosed air in the mesopores. The hydrophobicity of a film surface can be conveniently quantified by the traditional contact angle measurement. However, this technique cannot be readily applied for small particles of submicrometers due to their much smaller diameters compared to that of a liquid droplet. To investigate the hydrophobicity of the single particle with different roughness, the gel trapping technique (GTT) combined with SEM measurement was employed in this study. Previously, GTT has been developed and used to precisely measure the contact angle of nanoparticles with various sizes, even those smaller than 50 nm, followed by SEM or atomic force microscope observation. Figure 4 shows the high-resolution SEM images of the CL-MMS-0.6 (Figure 4a), CL-MMS-1.0 (Figure 4b), CL-MMS-2.5 (Figure 4c), and smooth MMS microspheres (Figure 4d) on the PDMS layer obtained through GTT from decane–water interfaces. It can be seen that individual CL-MMS microspheres were mostly embedded in the PDMS, while MMS is clearly visible (i.e., as a bulge) on the PDMS surface. The average contact angle values were calculated to be 136.4° ± 5° (n = 5) for CL-MMS-0.6, 127.5° ± 5° (n = 5) for CL-MMS-1.0, 107.7° ± 5° (n = 5) for CL-MMS-1.9, and 75.1° ± 5° (n = 5) for MMS. It indicates an enhanced hydrophobicity with the decreasing size of mesoporous silica “bumps” deposited on the core particles, which is consistent with the reported hydrophobic film.

To gain more insight about the formation process of CL-MMS, a detailed time-dependent study was conducted by withdrawing the intermediate samples at different stages of the reaction solution of CL-MMS-1.0 at 20 °C for quick observation under TEM. As shown in Figure S6, well-retained Fe₃O₄@RF microspheres with silica-CTAB composite nanoparticles (∼15 nm) attaching on the surface were observed after reaction for 30 min (Figure S6a1). It is mainly attributed to the amphiphilic CTAB molecules which act as a bridge to interact with both the RF surface via the hexane-mediated hydrophobic interaction and silica oligomers via electrostatic attraction, and furthermore, it can promote the co-assembly of CTAB and silica on the surface of Fe₃O₄@RF microspheres (Figure S6b1). After reaction for 2 h, numerous nanospheres with larger size (∼50 nm) were deposited on Fe₃O₄@RF microspheres, suggesting that more nucleation sites were generated for the
formation and growth of more nanospheres on the RF shell (Figure S6a2, b2). After 5 h of reaction, the small islands on the Fe₃O₄@RF microspheres surface were found to grow into large bumps, forming a thick mesoporous silica shell with a rough surface (Figure S6a3). It implies a continuous growth of mesostructured silica-CTAB nanocomposites starting from the nucleation sites on the Fe₃O₄@RF microspheres’ surface (Figure S6b3). By further prolonging the reaction time to 8 h, cauliflower-like core—shell microspheres with a mesoporous shell thickness of ~100 nm were obtained (Figure S6a4, b4).

To elucidate the formation mechanism of CL-MMS microspheres with different roughness, we proposed a kinetics-controlled surface coating process for the nano-engineering of a rough shell of mesoporous silica on of Fe₃O₄@RF microspheres (Figure 5). At the beginning, Fe₃O₄@RF microspheres were well dispersed in aqueous solution containing CTAB and ammonia. Then, the hexane solution containing TEOS was dropwise added and adsorbed on the interface of Fe₃O₄@RF microspheres due to the hydrophobic interaction between hexane and RF polymer. As is well-known, the formation of silica networks from TEOS consists of hydrolysis and condensation steps. The kinetics of hydrolysis and condensation can be controlled by the concentration of ammonia. The precursor TEOS in the hexane droplets adsorbed on the Fe₃O₄@RF microspheres was hydrolyzed at the hexane—water interface by ammonia-water, giving rise to negatively charged silica oligomers which can co-assemble with CTAB to form mesostructure nanocomposites. Notably, the introduction of n-hexane is necessary in this study, because it provides an oil—water interface for the controllable generation of mesostructured organic—inorganic nanocomposites. Other nonpolar solvents, such as benzene, toluene, and n-heptane, can also be used to replace n-hexane. When a small fraction of ammonia was used in the synthesis (Figure 5, route 1), the hydrolysis and condensation rate of TEOS is quiet slow, resulting in a relatively low concentration of silica-CTAB nanocomposites, but higher than the critical concentration for heterogeneous nucleation. It leads to the deposition of numerous mesostructured nanospheres on the RF shell which further grow into large mesoporous silica spheres as the reaction proceeds, forming CL-MMS-0.6 microspheres with a rough surface. Upon slightly increasing the amount of ammonia to 1.0 vol % (Figure 5 route 2), the hydrolysis and condensation of TEOS can be promoted, which causes a quick nucleation of silica-CTAB composite nanoparticles on RF shell which further grows into interconnected large particles on Fe₃O₄@RF microspheres, forming CL-MMS-1.0 microspheres with a less rough surface. By further increasing of the amount of ammonia to 1.9 vol %, the formation process is similar to Figure 5, route 2 except that the reaction kinetics is greatly enhanced. As a result, the hydrolysis and condensation rate of TEOS is increased extraordinarily high, causing a fast nucleation of silica-CTAB nanoparticles in a short time both on Fe₃O₄@RF microspheres and in the reaction solution. Meanwhile, the early stage generated silica-CTAB nanoparticles with a low cross-linking degree in the basic water phase constantly undergo dissociation and deposition on the RF shell, leading to the thick interconnected rough silica shell (CL-MMS-1.9). If the initial content of ammonia is too high (Figure 5, route 3), the reaction kinetics becomes so fast that the silica polyhedron was formed similar to previous reports regarding the formation of polyhedron mesoporous silica particles in high-alkaline conditions.32 As a result, a shell of thick polyhedron-like mesoporous silica was formed on the Fe₃O₄@RF microspheres. It is worth noting that hydrophilic silica-coated magnetite particles can also be used to synthesize CL-MMS microspheres due to their good affinity for CTAB-silica nanocomposites, while colloids with a hydrophobic surface is not suitable for the synthesis of composite mesoporous microspheres with rough surface due to the poor interface interaction between hydrophobic colloids and hydrophilic CTAB-silica nanocomposites.

Oxygen-free operation of sensitive chemicals or reagents is important in many fields including the pharmacy industry, chemical processing, and bioengineering. The noninvasive manipulation of solutions of chemicals that are sensitive to oxygen is particularly important and highly desired for many applications. In this study, inspired by the unique surface morphology and the enhanced hydrophobicity of the CL-MMS microspheres, we demonstrate the application of this new kind of functional microsphere for oxygen-free manipulation of aqueous solution droplets in a hydrophobic solvent using an applied magnetic field. First, 0.5 mL of phenol aqueous solution (0.2 M) containing 0.01 mg of CL-MMS-1.0 microspheres was injected into petroleum ether (20 mL) in a polyvinyl chloride (PVC) soft tube (inner diameter ~3.0 mm) which was horizontally placed, and the two ends were sealed. The aqueous solution forms a spherical brown droplet suspension (like a Pickering emulsion) with CL-MMS microspheres located at the oil—water interface due to their hydrophobic properties from the rough surface (Figure 6a). Then, 0.3 mL of ferric nitrate aqueous solution (0.05 M) was injected into petroleum ether at a distance of 10 cm away from the phenol/rough microspheres suspension. It forms a cylindrical yellow aqueous droplet in the tube (Figure 6b). By applying a magnet (8.0 × 10⁴ A/m) along the outside of the tube, the phenol aqueous solution wrapped by magnetic microspheres can be easily manipulated to swim back and forth (speed: ~5.0 mm/s) in the organic solvent and finally collide with ferric nitrate solution, forming violet Fe₃(C₆H₅O)₆³⁺ ions (Figure 6c, see Movie S3). Despite the simple demonstration of the rough magnetic microsphere-assisted droplet collision for complexation reaction, it is expected our CL-MMS microspheres can be used as nanocarriers of other aqueous solutions without oxygen contact for site-specific delivery, liquid sealing, or clogging and so on.

Moreover, recent reports have revealed that the surface roughness of particles can significantly affect their interactions with cell membranes due to their analogous pollens or virus structure. Rough nanoparticles exhibit promising enhanced adhesion for “hairy” objects and superior cell uptake properties.30–32 The Liu group33 has investigated the respective contribution of particle geometries toward relevant biological...
functions and found that rough staggered lamellae nanostructures had the fastest cellular uptake rate. In this work, the obtained CL-MMS microspheres possess a tunable surface roughness which is expected to achieve a fast cellular uptake rate. To investigate their potential biological applications, both rough CL-MMS and smooth MMS microspheres were examined by incubation with Pan02 cells.

To track the location of microspheres, they were first modified with FITC according to the procedure reported previously. Then, the microspheres with the same diameter (≈680 nm) and concentration (2.0 wt %) were incubated with Pan02 cells and observed by CLSM during the time course from 0 to 4 h incubation. As shown in Figure 7, the relative cell uptake rate was quite different between the two types of microspheres. Green fluorescence was observed in Pan02 cells for CL-MMS-1.0 microspheres after 0.5 h incubation, indicating the effective cell uptake properties. With prolonging the incubation time to 4 h, the fluorescence intensity in the cells was gradually enhanced, indicating a continuous cellular uptake process (Figure 7a). However, the smooth microspheres were observed in the cells with weak fluorescent intensity after 4 h incubation, indicating a relatively low cellular uptake rate. It confirms that the rough CL-MMS microspheres exhibit a faster cell internalization rate because nanoscale surface roughness can enhance the interaction of particles and cell membranes. Furthermore, considering the high porosity and radially aligned mesopores of the CL-MMS microspheres, these novel microspheres can find applications in fast drug delivery in cancer therapy.

- CONCLUSIONS

In summary, a new kind of cauliflower-like core–shell magnetic mesoporous silica microsphere with controllable surface roughness has been synthesized through the kinetics-controlled interface co-assembly and deposition of mesostructured nanocomposites on RF-coated magnetite particles. This robust interface nanoengineering enables a controllable nucleation of surfactant-silica nanoparticles on RF shells and the simultaneous growth of the mesostructured nanoparticles. The obtained microspheres possess well-defined core–shell structure with rough morphology, uniform sizes (560–1000 nm), open and perpendicularly aligned mesopore (~5.7 nm) channels as the outer shell, RF shell protected magnetic responsive core, high surface area up to 382 m²/g, and large pore volume of 0.66 cm³/g. The surface roughness and morphology, in other words, the mesoporous silica domain size on the RF surface, can be well tuned by kinetics-controlled interface coating process via adjusting the amount of basic catalysts. The obtained CL-MMS microspheres exhibit increasing hydrophobicity with the decreasing of the surface silica domain size. Thanks to their magnetic property and unique surface roughness, CL-MMS microspheres exhibit excellent performance in stabilizing and manipulating aqueous solutions in petroleum ether with the help of an applied magnetic field. The rough microspheres also exhibit superior cell uptake properties compared with traditional smooth core–shell magnetic mesoporous silica microspheres. Considering the open and accessible mesopore channels, it is expected that these novel microspheres may find application in fast drug delivery in cancer therapy.

- ASSOCIATED CONTENT

Supporting Information
The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.7b01464.

SEM and TEM images of Fe₃O₄ particles and Fe₃O₄@RF microspheres and smooth magnetic mesoporous silica (MMS) microspheres, SEM images, and XRD patterns of CL-MMS-1.0 microspheres; the magnetic hysteresis loops of Fe₃O₄ and CL-MMS-1.0 microsphere; TEM images of CL-MMS intermediates at different reaction stages (PDF)
Movie S1: the electron tomography imaging of CL-MMS microspheres (AVI)
Movie S2: the cross-sectional TEM imaging of CL-MMS microspheres (AVI)
Movie S3: The magnetic field guided movement of the aqueous droplet stabilized by CL-MMS microspheres (AVI)

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