General one-pot strategy to prepare multifunctional nanocomposites with hydrophilic colloidal nanoparticles core/mesoporous silica shell structure

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**Abstract**

A general and facile strategy was developed to coat hydrophilic inorganic nanoparticles directly with mesoporous silica nanoparticles (MSNs). The cationic surfactant of cetyltrimethylammonium bromide (CTAB) was adsorbed to various negatively charged CdTe quantum dots, Fe\textsubscript{3}O\textsubscript{4} nanocrystals or Au nanoparticles, introducing the bilayer of CTAB overcoating with positive charge. The subsequent sol–gel reaction of TEOS with the basic catalyst resulted in uniform nanocomposites. The concentration of CTAB and NH\textsubscript{4}OH in the recipe strongly influenced the number of inorganic nanoparticles in the nanocomposites and the homogeneity of MSNs shell. One dimensional Au nanorods and larger size of solid SiO\textsubscript{2} nanoparticles were also able to coat with MSNs using a similar synthetic procedure. The proposed method was greatly simplified without the help of any mediators or silane coupling agents and excellent mesostructural performance was readily achieved. Compared to the methods known from the literatures for the coating of hydrophobic nanoparticles, this efficient way is especially useful for trapping different hydrophilic nanoparticles with arbitrary sizes and shapes into MSNs. These highly versatile multifunctional nanocomposites, together with the pH-responsible drug release behaviors, non-toxicity to normal cells and ease of uptake into cancer cells, are expected to be utilized as drug delivery system for simultaneous imaging and therapeutic applications.

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1. Introduction

In recent years, a wide variety of strategies for the advanced high-quality synthesis of silica-coated inorganic colloidal nanoparticles have emerged at the forefront of materials science [1,2]. Silica as a shell material promises an unparalleled opportunity for enhancement of colloidal properties and important functions including ease of synthesis, high biocompatibility and controllable porosity [3]. To date, extensive studies have witnessed rapid advances in the elaborate synthesis of two major types of silica-based materials, including solid silica nanoparticles and mesoporous silica nanoparticles (MSNs) [4,5]. As an alternative to hybrid nanocomposites constructed from solid silica materials, the burgeoning interests on MSNs-based systems have been greatly spurred, due to the unique properties for MSNs such as high surface area, pore volume and prominent biocompatibility [6–9].

The ability to encapsulate inorganic colloids by growing mesostructured silica around them introduces the intrinsic functionality of the nanoparticles and at the same time enables the storage of guest molecules inside of the mesoporous channels [10–12]. Several preparation techniques were documented to incorporate inorganic nanocrystals into the mesoporous silica matrix. Back filtration of metal salt solutions into the mesoporous silica channels and subsequent in situ formation of metal nanoparticles had been applied to synthesize inorganic nanoparticles-embedded MSNs composites [13]. However, the metal nanoparticles in the pore channels resulted in the blocking of pores and had detrimental effects on the access of guest molecules into the mesopores. Tumbler-like ordered mesoporous silica nanocomposites were synthesized in the presence of pre-existing solid silica-coated magnetic nanocrystals [14]. Pt nanocrystals stabilized with the polymer of poly(vinylpyrrolidone) were successfully coated with mesoporous silica shells via the sol–gel reaction [15]. Although the use of intermediate layers can arguably form the basis of a common mesoporous silica-coating procedure, the successful integration of as-synthesized inorganic nanoparticles into MSNs was not trivial because it was generally involved tedious surface treatment and a special process to confine the region in which the hydrolysis and condensation reaction of TEOS occur. Recently, one-pot synthesis protocol was presented to coat an individual hydrophobic magnetic core with a layer of mesoporous silica shell [16,17]. This useful synthetic protocol can be generalized to
produce core/shell nanocomposites composed of a single hydrophobic inorganic nanoparticles core and mesoporous silica shell. In our previous investigation, the synthesis of magnetic delivery vehicles by encapsulating hydrophobic Fe₃O₄ nanocrystals in nano-sized MSNs was also reported [18]. Actually, water-dispersible nanoparticles are indispensable for various biomedical applications. From the view of producing new materials for clinic use, it is of great interest to design and synthesize the multifunctional nanocomposites with hydrophilic inorganic nanocrystals as core and MSNs as shell. Nonetheless, preparations of these nanocomposites are still not straightforward because of most of the good synthetic methods available for hydrophobic inorganic nanoparticles. It is necessary for a detailed and systematic investigation of the interdependent synthesis parameters (e.g., amount of CTAB, pH value, and kind of colloidal seeds) on the size and morphology of the resulting nanoscaled nanocomposites. The development of a general approach for the fabrication of a variety of water-dispersed inorganic nanoparticles directly coated into MSNs matrix is a great challenge.

In the work presented here, a novel avenue to create a general approach was explored for the fabrication of core/shell architectural nanocomposites, wherein hydrophilic inorganic nanoparticles as core and MSNs as outer shell. The cationic surfactant of CTAB was adsorbed to various negatively charged inorganic nanoparticles including CdTe quantum dots, Fe₃O₄ magnetic nanocrystals or small Au colloids, introducing a positive charge bilayer of CTAB-stabilized inorganic nanoparticles. The subsequent sol–gel reaction of TEOS in the aqueous solution with the basic catalyst can be directly resulted in the core/shell-structured nanocomposites. To the best of our knowledge, in spite of a number of publications on silica-based nanocomposites appeared in the last years, little interest has been devoted to the rationally design of nanocomposites based on MSNs coated hydrophilic inorganic nanoparticles directly, without requiring other modification procedures. The method had also been successfully used to coat mesoporous silica shell on Au nanorods with one dimensional nanostructures and larger size of solid SiO₂ nanoparticles (40 ± 5 nm in diameter). As anticipated, the strategy here may be useful in the synthesis of a wide range of core/shell nanocomposites incorporating inorganic nanoparticles with different characters, such as positively or negatively charged surface and spherical or rod shapes. The results elucidated the predominant factors that determine the morphology of resulting core/shell materials, which will then provide practical guidance for rationally designing nanocomposites as biomedical devices with multifunctional functions. The performances for nanocomposites, such as the cytotoxicity to normal cells, the uptake by cancer cells and the drug release behaviors, were also specifically evaluated.

2. Experimental section

2.1. Materials

Iron (III) chloride hexahydrate (FeCl₃·6H₂O, 97+%), cadmium dichloride (CdCl₂, 99%) and 3-mercaptopropionic acid (MPA) (98%) were purchased from Acros. Te powder (99.8%), chloroauric acid (HAuCl₄·C₁₂H₂O₆, 99.9%), sodium borohydride (NaBH₄, 99%), silver nitrate (AgNO₃, 99+%) and ascorbic acid (99+) were obtained from Aldrich. Iron (II) chloride tetrahydrate (FeCl₂·4H₂O, 99%) and sodium citrate (99%) were purchased from Fluka. Sodium hydroxide (NaOH), tetraethyl orthosilicate (TEOS), cetyltrimethylammonium bromide (CTAB), ethyl acetate (EA), NH₄OH (25% NH₃ in water) and ammonium nitrate (NH₄NO₃) were analytical grade and commercially available products. All the above chemicals were used without any additional purification, and deionized water was used in all the experimental procedures.

2.2. Characterization

The quantum dots were visualized using a FEI Tecnai F20 transmission electron microscope (HRTEM) operating at a voltage of 200 kV. The investigations of other products were performed on a JEOL 1230 TEM. Samples were prepared on a carbon-coated copper grid by evaporating one drop of diluted aqueous suspension of the nanoparticles. The size distribution of the nanoparticles was measured by dynamic light scattering (DLS) using an autozizer 4700 (Malvern). Zeta potential was measured by Zetasizer Nano ZS (Malvern) at 25 °C. Thermogravimetric analysis (TGA) was carried out on a Perkin-Elmer Lambda 35 spectrophotometer. Nitrogen adsorption–desorption isotherms were obtained on a Micromeritics Tristar 3000 pure analyzer at 77 K under continuous adsorption conditions. Brunauer, Emmett, and Teller (BET) and Barrett, Joyner, and Halenda (BJH) analysis was used to calculate the surface area, pore size and pore volume. Low-angle X-ray diffraction (XRD) patterns were recorded on a Bruker D4 X-ray diffractometer with Ni-filtered Cu KR radiation (40 kV, 40 mA). The cellular images were acquired with a confocal laser scanning microscope (CLSM, LEICA TCS S5P II).

2.3. Synthesis of CdTe@MSNs nanocomposites

The preparation MPA-capped CdTe nanocrystals had been described in detail by the hydrothermal route according to our previous paper with some modifications [19]. The molar ratio of CdCl₂·2·Mg·NaHTe was fixed at 1:1.80·0.125 for all samples. In brief, 0.1 g of NaBH₄ was dissolved in 5 mL deionized water at 4 °C (the ice-water bath) and then 0.158 g of Te powder was added. With continuous N₂ flow and vigorous stirring, a fresh solution of NaHTe was produced after 12 h. Meanwhile, the stock solution of CdCl₂ with MPA was prepared. MPA was added into CdCl₂ aqueous solution, and the pH was adjusted to 9.0 by using 0.1 M of NaOH to form a solution of Cd precursor with a concentration of 10 mM. Then NaHTe solution was rapidly injected into the above N₂-saturated stock solution under vigorous stirring. Finally, 9 mL of the mixed precursor solution was put into a Teflon-lined stainless steel autoclave and maintained at 185 °C for a certain time, then cooled to room temperature by hydro-cooling process. The reaction time of 40, 50, 60 and 75 min gave four size factions of CdTe nanocrystals with green, orange, red and near-infrared emission color, respectively.

The CdTe@MSNs nanocomposites were synthesized via the base-catalyzed sol–gel method in the existence of CdTe nanoparticles acted as seeds for the packing and self-assembly of silica-surfactant complexes, under different concentrations of CTAB: 1, 2 and 3 mg/mL. A typical procedure can be briefly described in the following way. A 5 mg of CdTe nanocrystals, 0.2 g of CTAB and 2 mL of NH₄OH were mixed in 100 mL aqueous solution under vigorous stirring. The mixed dispersion was homogenized for 3 h to form a uniform dispersion at 40 °C. Then, 0.5 mL of TEOS and 5 mL of ethyl acetate were successively added with rapid stirring for 1 min and kept at 80 rpm for another 6 h at 40 °C. The synthesized product was centrifuged, washed with distilled water and ethanol for several times. In order to remove the structure-directing agent of CTAB from the mesopores, an ion exchange procedure was used [20]. The as-synthesized materials were dissolved in a solution of NH₄NO₃ in anhydrous ethanol (10 mg/mL). The resulting mixture was heated to reflux and kept at that temperature.
for 30 min. After it was collected by centrifugation and washed with ethanol repeatedly, the obtained sample, designated as CdTe@MSNs-X \((X = 1, 2 \text{ and } 3 \text{ mg/mL of CTAB concentration in the recipe})\), was then redispersed in deionized water.

2.4. Synthesis of other typical nanocomposites

2.4.1. \(\text{Fe}_3\text{O}_4\) nanoparticles

The preparation of citrate-capped \(\text{Fe}_3\text{O}_4\) nanoparticles was synthesized using the described method with minor modification \([21]\), based on the co-precipitation. In a typical reaction, 5.2 g of FeCl\(_3\)·6H\(_2\)O and 2.0 g of FeCl\(_2\)·4H\(_2\)O were mixed with 25 mL of concentrated ammonia aqueous \((25 \text{ wt.}\% )\) at 90 °C under \(\text{N}_2\) flow. The obtained magnetic nanoparticles were then washed with deionized water and dispersed in 100 mL of sodium citrate solution \((1.0 \text{ M})\) by ultrasonication. The mixture was stirred for 12 h at 60 °C, and the magnetic nanoparticles were washed with anhydrous ethanol and redispersed in deionized water to obtain a magnetic fluid of 5 wt.% for further use.

2.4.2. Au nanoparticles

Colloidal small Au nanoparticles with a mean diameter of 15 nm were prepared according to the standard sodium citrate reduction method \([22]\). A total of 500 mL of a boiling solution of 1 wt.% HAuCl\(_4\)·3H\(_2\)O in deionized water was reduced with 3 mL of a solution of sodium citrate \((0.47 \text{ mg/mL})\). Deep-red dispersion was used without further purification.

2.4.3. Au nanorods

For preparation of Au nanorods, seed and growth solutions were made as described below \([23]\). CTAB solution \((5 \text{ mL, } 72.9 \text{ mg/mL})\) was mixed with 5.0 mL of 0.2 mg/mL HAuCl\(_4\)·3H\(_2\)O. Then, 0.6 mL of 0.38 mg/mL NaBH\(_4\) was added at 0 °C with vigorous stirring and continued for 2 min, which resulted in the formation of a brownish yellow solution. After the solution was stirred, it was kept at 25 °C. The growth solution was prepared by stirring together 5 mL of 72.9 mg/mL CTAB, 0.25 mL of 0.68 mg/mL AgNO\(_3\), 5 mL of 0.2 mg/mL HAuCl\(_4\)·3H\(_2\)O. Then, gentle mixing of the solution, 70 μL of 13.9 mg/mL ascorbic acid was slowly added. Finally, 12 μL of seed solution was added to the growth solution at 27–30 °C. The entire solution was mixed and left undisturbed overnight \((14–16 \text{ h})\). The brown colored Au nanorods dispersion was purified by centrifugation to remove excess CTAB \((\text{twice at } 14,000 \text{ rpm, 5 min each})\).

2.4.4. Solid SiO\(_2\) nanoparticles

A mixture of ethanol \((50 \text{ mL})\) and deionized water \((10 \text{ mL})\) was mixed drastically. After addition of NaOH solution \((1.25 \text{ mL})\), the precursor of TEOS \((0.5 \text{ mL})\) was added to the reaction solution with mechanical stirring at 40 °C for 12 h. The preformed SiO\(_2\) nanoparticles solution with light-blue color was stored without any purification.

For the synthesis of nanocomposites with different core colloids, the proposed simple method can be applied to the above as-prepared nanoparticles regardless of their chemical compositions, shapes and sizes. The obtained samples were named as Fe\(_3\)O\(_4@\)MSNs, Au@MSNs, Au nanorod@MSNs and SiO\(_2@\)MSNs, respectively.

2.5. Drug loading and release

Doxorubin hydrochloride (DOX) was applied as a model drug, and the behaviors of drug storage/release were investigated. The DOX solution \((1 \text{ mg/mL})\) in deionized water produced a pH about 5.3. Aqueous NaOH solution \((0.1 \text{ M})\) was added to this DOX solution to reach the desired pH of 8.5. A 5 mg of CdTe@MSNs was ultrasonically dispersed in 1.5 mL of DOX solution. The mixture was stirred at 25 °C for 12 h. Then the dispersion was centrifuged to collect the DOX-loaded CdTe@MSNs and washed with deionized water for twice to remove the physical adsorbed DOX. The mass of DOX in the carrier was calculated by subtracting the mass of DOX in the supernatant from the total mass of drug in the initial solution by UV–vis at 485 nm.

For the various pH release studies, three phosphate buffer solutions \((0.15 \text{ M})\) were chosen with different pH values of 5.0, 6.5 and 7.4. DOX-loaded CdTe@MSNs was dispersed in 2 mL of buffer, and the dispersion was transferred into dialysis bag (molecular weight cut off 14,000). The dialysis bag was then kept in 200 mL of buffer and gently shaken at 37 °C. At timed intervals, 1 mL of solution was withdrawn from the dispersion periodically and analyzed by UV–vis. For keeping constant volume, 1 mL of fresh buffer was added after each sampling. All drug release results were averaged with three measurements.

2.6. In vitro cell assay

HEK 293 \((\text{Human Embryonic Kidney})\) normal cells, rat basophilic leukemia (RBL) cancer cells and HeLa cancer cells were cultured in DMEM medium supplemented with 10% \((\text{v/v})\) fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, 100 μg/mL 10 streptomycin in 37 °C, 5% CO\(_2\). For all experiments, cells were harvested from sub-confluent cultures by the use of trypsin and were resuspended in fresh complete medium before plating.

The cytotoxicity assay of blank CdTe@MSNs against 293 and HeLa cells was assessed by using the standard 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay. The 293 cells were then seeded in 96-well plates at the density of 5000 viable cells per well and incubated 24 h to allow cell attachment. Then the cells were incubated with blank CdTe@MSNs at the indicated concentrations, respectively. After 24 h, the medium were replaced with fresh DMEM containing MTT \((5 \text{ mg/mL})\), and 293 cells were incubated for additional 4 h. Upon the removal of MTT solution, the purple formazan crystals were dissolved with DMSO, and the absorbance was monitored at 570 nm on a micro-plate reader (FL600, Bio-Tek). The results were expressed as mean values of three measurements. The same process of cytotoxicity analysis of pure nanocomposites to HeLa cells was implemented as aforementioned.

The near-infrared CdTe@MSNs nanocomposites were used to study the cell uptake by the RBL and HeLa cancer cells. In a typical procedure, \(1.0 \times 10^5\) mL\(^{-1}\) cancer cells were seeded onto the glass cover slips in a 24-well plate in DMEM medium containing 10% fetal bovine serum for 24 h to allow the cells to attach. The medium was then replaced with 1 mL culture serum-free medium containing CdTe@MSNs nanocomposites \((100 \mu\text{g/mL})\). After incubation for 4 h, the cell monolayer on the cover slip was repeatedly washed with PBS to remove the remaining nanocomposites and then sealed with a microscope glass slide. Observations were monitored using the CLSM.

3. Results and discussion

3.1. Coating of hydrophilic CdTe nanocrystals

The detailed discussion of our method was presented for coating of hydrophilic luminescent CdTe nanocrystals, because generally mesoporous silica shell cannot be grown directly on such nanoparticles. Moreover, fluorescent labeling has a great potential for disease therapy, as a consequence of the fact that it can be easily identified and tracked \([24]\). A typical procedure for the fabrication of CdTe nanocrystals embedded into mesoporous silica
nanoparticles (CdTe@MSNs) was illustrated in Scheme 1. As-prepared CdTe nanocrystals were capped by hydrophilic MPA ligands through the hydrothermal route [19], thus providing negatively charged CdTe quantum dots dispersed in aqueous solution. Most often, with regard to biological applications, the types of inorganic nanoparticles are required to be stable and dispersible under aqueous biological conditions with negatively charged character [25]. Therefore, to conduct the sol–gel reaction for yielding mesoporous silica coatings directly outside of the CdTe nanocrystals, it was of paramount importance to adjust the chemistry of the surfactant head groups on the surface of the CdTe seeds, which can fit the requirement of transferring these hydrophilic CdTe nanocrystals from negatively charge to positively charge allowing to the tightly bound bilayer of cationic CTAB surfactants. Then, a silica precursor TEOS hydrolyzed, condensed and produced a cooperative bilayer of cationic CTAB surfactants. Then, a silica complex by $S^+I^- \text{ (S: surfactant; I: inorganic species)}$ pathway under basic conditions [26]. In addition, the number of CdTe nanocrystals overcoated with CTAB surfactants can be controlled by changing the relative amount of CTAB and the CdTe seeds. A higher CTAB/CdTe mass ratio would be expected to lead to the formation of less incorporated CdTe quantum dots in MSNs matrix. The obtained CdTe@MSNs with a highly ordered mesostructure was subsequent treatment with fluxing NH$_4$NO$_3$ ethanol solution in order to remove CTAB organic templates [20]. This simple method of synthesizing CdTe@MSNs can be carried out in a one-pot system without necessitating isolation or purification steps.

Invariably, it was vital to keep a repulsive force between the CdTe nanocrystals to stabilize their colloidal suspension, even during the CTAB overcoated step or mesoporous silica shell-coating procedure. The interaction between the adsorption of cationic CTAB surfactants and the negatively charged CdTe nanocrystals was monitored using zeta potential at pH 11.7 (shown in Fig. 1). The zeta potential of the pure CdTe quantum dots was $-35.3$ mV, indicating that the as-prepared MPA-capped CdTe nanocrystals were negatively charged due to the stabilizer. It became evident that with increasing CTAB concentration, the zeta potential values were found to have a monotonous increase and approach a plateau close to $40$ mV beyond the CTAB concentration of $0.6$ mg/mL. At pH 11.7, the net balanced charge on the surface of the nanoparticles to keep them apart. For the system with lower zeta potential, the measurement was carried out within the short period. As the amount of CTAB increased to $0.6$ mg/mL, the available positive charge increased along with it, and the MPA ligands were eventually offsetted by the bilayer of CTAB surfactants. Consequently, $2$ mg/mL of CTAB overcoated CdTe nanocrystals were highly stable in dispersion as indicated by the high zeta potential values of $40$ mV, and no visual flocculation and sedimentation were observed after the CTAB coated process.

The observation of the stability of CTAB overcoated CdTe nanocrystals as a function of CTAB concentrations was interesting. At first, the MPA-capped CdTe nanocrystals were well-dispersed in deionized water at pH 11.7 (the left inset of Fig. 1). The attractive electrostatic force was employed to adsorb the first layer of cationic CTAB surfactants onto the negatively charged CdTe quantum dots [27], introducing a partial hydrophobic character to the surface of CdTe due to the effect of 16-carbon tail of CTAB. As a result, these CdTe nanoparticles were stable in CHCl$_3$ solvent (the middle inset of Fig. 1). Upon increase in CTAB concentration, CdTe nanoparticles were transferred to an aqueous phase driven by the van der Waals interactions between the hydrophobic chains of the first layer and the second layer of CTAB surfactants (Scheme 1), leading to the formation of the bilayer structures with quaternary ammonium groups pointing outwards [28]. Consequently, as visualized in the right inset of Fig. 1, the bilayer of CTAB-stabilized CdTe nanocrystals maintained well-dispersed in deionized water once again.
As proposed in Scheme 1, after the CTAB surfactants were added, the CdTe nanocrystals were coated with well-organized CTAB molecules. This hypothesis was supported by TGA data. Quantitative determination of the CTAB content bound on the surface of CdTe nanoparticles was also executed (Fig. 2). For primary MPA-capped nanoparticles, the weight loss (about 6.8 wt.%) could be observed over a temperature range of 280–480 °C, which can be ascribed to the organic material of MPA ligand [29]. Furthermore, in the case of CTAB (2 mg/mL) overcoated CdTe nanocrystals, the TGA profile exhibited a sharp weight loss about 29.3 wt. % centered at 260 °C. This weight loss was due to the decomposition of total bound CTAB molecules with respect to the pure as-synthesized CdTe nanocrystals [30]. The primary results from TGA results might be indirectly induced the abundant CTAB surfactants onto the surface of CdTe quantum dots providing enough positive charge for the formation of mesoporous silica shell around the CdTe seeds.

Typical TEM images of the CdTe seeds before and after coating with mesoporous silica were presented in Fig. 3. The evolution of morphology and structure for the obtained surfactant-extracted CdTe@MSNs as a function of CTAB amounts were examined. The concentration of CTAB was increased from 1, 2 to 3 mg/mL in the synthesis recipe, indexed to the samples of CdTe@MSNs-1, 2, 3, while keeping all other reactant concentrations constant under the same conditions. The size of as-made CdTe nanoparticles, without any size selection, was dominant at 5 nm (Fig. 3a). Meanwhile, as shown in the Fig. 3b–d, the appearance of darker core was clearly observed in each particle, implying that CdTe nanoparticles were embedded in the center of mesoporous silica matrix successfully with obvious core/shell nanostructures [31]. In fact, the well-dispersed and uniform spherical nanocomposites were obtained for a range of CTAB concentration comprised between 1 and 3 mg/mL, without aggregation from one another. There was no significant difference in the size of CdTe@MSNs for all the three samples. The average CdTe@MSNs size of separated grain was approximately 130 ± 10 nm. Due to the small size of CdTe, the number of CdTe nanoparticles incorporated cannot be accurately determined from TEM [32,33]. Nonetheless, the results in Fig. 3 proved that the actual number of aggregated CdTe seeds encapsulated within MSNs matrix was more than one and generally decreased with the amount of CTAB increased. TEM images of CdTe@MSNs-1 (Fig. 3b) clearly showed that multiple aggregates of CdTe at the low CTAB concentration of 1 mg/mL, while most of CdTe nanoparticles formed dimers or trimers at the CTAB concentration of 2 mg/mL (CdTe@MSNs-2, Fig. 3c), and most of CdTe nanoparticles remained isolated at higher CTAB concentration (CdTe@MSN-3, Fig. 3d). The number of aggregated CdTe nanocrystals for the core clusters reflected the flocculation of CdTe seeds at the beginning of the experiment prior to silica mesophase growth [22]. CdTe clustering can be detected by a small red shift in the fluorescence emission peak of the colloidal CdTe nanocrystals. It should be mentioned that the concentration of CTAB had to be maintained within a certain range, since the empty and connected rod-shaped MSNs allowed to be produced if the concentration of CTAB was extremely high (5 mg/mL, data not shown). A higher CTAB concentration resulted in the less number of CdTe nanocrystals incorporated in nanocomposites. The origin of this effect was related to the increase in total surface area of aggregated CdTe nanocrystals while decreasing their cluster diameters [34]. The presence of excess CTAB surfactants ascribing to shorter rod micelles without the encapsulation of any seeds, which could be offered to the growing mesoporous silica, could explain the occurrence of pure MSNs.

To provide further experimental support for the number of CdTe seeds located in each MSN, the photoluminescence spectra were carried out to monitor the formation process of CdTe@MSNs. The results were shown in Fig. 4 and the inset analyzed the shift of corresponding fluorescence emission peaks. As-prepared water-dispersible CdTe nanocrystals exhibited an intense, narrow emission spectrum with a peak at 692 nm (Fig. 4a). It had been observed that after the addition of 1 mg/mL of CTAB, the complexes of CTAB (1 mg/mL) surfactants overcoated CdTe complexes retained a comparable intense and narrow fluorescence emission behavior, but with its peak position at 702 nm. This result was related to the strong interaction between the negative CdTe nanoparticles and the cationic CTAB surfactants [35]. In addition, the bilayer of CTAB molecules also changed the polarity of the microenvironment surrounding the CdTe nanocrystals. Bathochromic shift was associated with the aggregation extent of CdTe seeds, as a result of the energy transfer [36]. Consequently, the increase in the number of CdTe nanocrystals encapsulated within the complexes led to more red shift. Then, once the mesoporous silica shell was grew in situ outside of the CTAB overcoated CdTe complexes directly through the Coulomb forces interaction (‘S’1’), the fluorescence spectrum had further red shift to 715 nm for CdTe@MSN-1 (Fig. 4c). As previously reported, the refractive indices of water solution and the silica shell were set as 1.33 and 1.45, respectively [37]. The origin of this shift could be explained by the change in the refractive index of the medium surrounding CdTe after mesoporous silica encapsulation (larger than that of water), because the number of CdTe located in MSNs was the same as the CTAB overcoated CdTe complexes. The resulting partly decreased fluorescence intensity was due to the thick mesoporous silica shell or the larger size of nanocomposite [38]. With an increase in the concentration of CTAB, the obvious attenuation of fluorescence intensity was measured for the samples of CdTe@MSNs-2 and CdTe@MSNs-3 (Fig. 4d and e). This phenomenon had been considered as changes of the sizes of CdTe encapsulated in the nanocomposites rather than changes of the size of CdTe@MSNs. As shown in Fig. 3b–d, the diameters were nearly unchanged for all the obtained nanocomposites, irrespectively of the concentration of CTAB from 1 to 3 mg/mL. Interestingly, according to the trend illustrated in the inset of Fig. 4, it was found that further increasing the CTAB concentration in the recipe produced a decreasing extent of the wavelength red shift. The emission peaks were 711 and 709 nm, corresponding to the nanocomposites of CdTe@MSNs-2 and CdTe@MSNs-3, respectively.

Herein we proved that the number of aggregated CdTe nanocrystals encapsulated within MSNs matrix was decreased in response to the increase in the CTAB concentration. This finding can be rationalized in the following scenario. It appeared that at low CTAB/CdTe ratios, smaller primary particles with fewer
associated surfactant tails were formed, which had a weaker dispersion potential toward self-assembly [22]. As a result, the CTAB overcoated CdTe seeds were free to flocculate. Higher CTAB/CdTe ratios, as were exhibited by samples of CdTe@MSNs-2 and CdTe@MSNs-3, formed larger numbers of primary particles, with a correspondingly greater dispersion potential through positive charge repulsion. Hence, these seeds could not flocculated, leading to the lower cluster numbers. A combination of the above results (Figs. 3 and 4) suggested as the concentration of CTAB used in the synthetic process increased, the resulting number of CdTe seeds coated into the mesoporous silica shell decreased, making it easy to precisely regulate the overall fluorescence intensity for the synthesized CdTe@MSNs nanocomposite.

It should be noted that the CTAB overcoated CdTe nanocrystals should not be stored too long in water (less than 3 days) before the mesoporous silica shell was coated. If the nanoparticles were placed for a longer time, the mesoporous silica shell was observed to be less homogeneous as shown by TEM measurements. The reason for this was probably a slow desorption of CTAB surfactants.

The synthesis of mesoporous silica required the participation of the template agent of CTAB, and TEOS was subsequently hydrolyzed and condensed using NH₄OH as base catalyst. In our further study, the concentration of CTAB was held at 2 mg/mL for the preparation of CdTe@MSNs nanocomposite because of the presentation of optimal morphology and highly stable suspension (Fig. 3c). For the thorough investigation of the effect of the initial reaction pH on the core/shell-structured nanocomposite within the nanoscaled size, the syntheses of CdTe@MSNs nanocomposites were carried out at several different pH values by simply changing the concentration of NH₄OH catalysts. The other steps were identical to what had been described in the foregoing synthesis step. It is known that the rate of hydrolysis of TEOS and condensation of Si–OH to form siloxane bonds are the two key synthesis parame-
The amount of NH$_4$OH reaction reagent (the inset in Fig. 5). Actually, CdTe@MSNs nanocomposites was controlled by changing the lysis reaction of TEOS. The fast deprotonation of protonated silanols resulted from hydrolysis of the total TEOS. When the highest pH value of starting reaction mixture: a low- pH system needed a longer time. The reaction was preceded for 6 h and was determined to have a final solution pH of 9.7. A substantial decrease in pH also took place during the reaction in the presence of other NH$_4$OH contents as shown in Fig. 5 (step 4 and step 5). For the other three samples, the pH values decreased from 11.2, 11.4 and 11.9 to 9.3, 9.5 and 9.8, respectively, after complete hydrolysis and condensation processes, the final CdTe@MSNs nanocomposites with discrete and uniform characters could be finely tuned by simply changing the pH value of starting reaction system.

In the fabrication procedure, the shape of the obtained CdTe@MSNs nanocomposites was controlled by changing the amounts of NH$_4$OH reaction reagent (the inset in Fig. 5). Actually, the increase in the NH$_4$OH concentration resulted to rapid formation of nanoparticles, thicker mesoporous silica shell and higher uniformity for the nanocomposite. It was worth mentioning that at the initial amount of 0.5 v/v% NH$_4$OH content, the reaction system did not turn any turbidity during the experiment process, which indicated that the amount of basic catalyst was far from sufficient for the hydrolysis of the total TEOS. When the highest pH 11.9 (NH$_4$OH content of 4.0 v/v%) was employed, the diameter of nanocomposites increased to approximate 150 ± 20 nm (the inset in Fig. 5d), but no well core/shell structures were observed. This was understandable because the hydrolysis rate of TEOS at higher pH was faster [41], thus leading to more primary silicate species in the system. These primary species in close vicinity generally self-assembled to form nuclei, which was seeded as the growth location in the next hydrolysis progresses [42]. Hence, empty mesoporous silica shell was inevitably produced if the concentration of the primary species was too high. At pH 11.7 (Fig. 5c), core/shell architectural CdTe@MSNs with the size of about 130 ± 10 nm were obtained. Our experiments had provided strong evidence that the initial pH value of the synthesis system was extremely main factor affecting the core/shell morphology of the products. These results encouraged us to consider that in order to control the proper rate of hydrolysis and condensation processes, the final CdTe@MSNs nanocomposites with discrete and uniform characters could be finely tuned by simply changing the pH value of starting reaction system.

To explore the generality of the fluorescence of CdTe@MSNs nanocomposites, we had investigated the behavior of various types of quantum dots that covered the distinct emission spectrum (Fig. 6). The core/shell architecture of CdTe@MSNs nanocomposite could be expanded to incorporate a wide range of other quantum dots by using different CdTe seeds in the core part, including green, orange, red and near-infrared quantum dots, respectively. Similar to the results obtained from Fig. 4a (pure CdTe quantum dots), other classes of CdTe nanocrystals also exhibited typical characteristics of fluorescence emission. The green, orange, red and near-infrared CdTe quantum dots produced distinct emission wavelengths at 535, 597, 652 and 715 nm, respectively. The fluorescence emission spectra of the various CdTe@MSNs nanocomposites changed accordingly to the incorporated quantum dots. The emission peaks were red-shifted (around 15 nm in these cases) compared to those of the pure CdTe nanoparticles in aqueous medium [19]. Under the illumination using a UV lamp (365 nm), digital photographs of an aqueous dispersion of CdTe@MSNs displayed remarkable and different fluorescent colors (inset of Fig. 5).

**Fig. 5.** Variation of pH values in each synthesis step for CdTe@MSNs-2 as a function of initial NH$_4$OH contents (v/v): (a) 0.5%; (b) 1.0%; (c) 2.0%; and (d) 4.0% and the corresponding TEM images in the inset.
developed mesoporous structure, with a sharp capillary condensation.

CdTe@MSNs exhibited the characteristic IV behavior for a well-ordered 2D hexagonal mesoporous structure [6]. As shown in Fig. 7b, the nitrogen adsorption/desorption isotherms for (BET) surface area and the total pore volume were 960 m$^2$/g and 1.4 cm$^3$/g, respectively. A narrow pore size distribution of 2.8 nm was obtained for mesopores using the Barrett–Joiner–Halenda (BJH) method (Fig. 7c). Finally, to evaluate the colloidal stability in deionized water, DLS result in Fig. 7d revealed that 130 ± 10 nm diameter (TEM) CdTe@MSNs had a hydrodynamic diameter of 160 nm in deionized water, and a narrow size distribution verified by the small values of PDI (0.08). The synthesized nanocomposites remained well-dispersed without any visible aggregation after room temperature storage for 2 weeks. The slightly size difference between TEM and DLS measurements was attributed to the hydration nanocomposites [43]. The well-ordered multifunctional CdTe@MSNs with large surface area, visible to near-infrared fluorescence and stable in aqueous systems could be used as drug delivery systems. Furthermore, the in vitro performance of the blank nanocomposites to normal and cancer cells would be discussed exhaustively below.

3.2. Coating of other hydrophilic nanoparticles

Similarly, the proposed bilayer of CTAB surfactant-overcoated CdTe nanocrystals method could be applied to other kinds of hydrophilic nanomaterial with different chemical components and shapes. The as-made Fe$_3$O$_4$ magnetic nanocrystals, Au nanoparticles and solid SiO$_2$ nanoparticles were negatively charged, whereas the Au nanorods were positively charged. The zeta potentials of these inorganic nanocrystals were −20.8 mV, −23.2 mV, −31.1 mV and 34.6 mV at pH 7.4, correspondingly. Fig. 8 showed TEM images of these inorganic nanoparticles before and after coating with mesoporous silica shells. For example, the nanocrystals of Fe$_3$O$_4$ and Au (Fig. 8a and c) acted as seeds for the growth of more homogeneously sized mesoporous silica shells. The average sizes of the Fe$_3$O$_4$@MSNs and Au@MSNs nanocomposites were 150 ± 10 nm and 140 ± 15 nm, respectively. Several Fe$_3$O$_4$ nanoparticles and only one Au nanoparticle were embedded in each nanocomposite, respectively.

To test the generality of our method, we also coat mesoporous silica shell on one dimensional nanostructure of Au nanorods and larger size of solid SiO$_2$ (40 ± 5 nm). In this case, Au nanorods (Fig. 8e) were coated with a distinct mesoporous silica shell (Fig. 8f) using a very similar synthetic procedure to that employed for the spherical nanoparticles. It should be paid attention that to coat these nanorods with uniform mesoporous silica shell, a higher CTAB concentration of 3 mg/mL was necessary to adsorb onto the nanorods because of their larger size (length 40 nm, width 15 nm). Then, the silica source of TEOS was carried out at a lower injection rate within 30 min interval to prevent aggregation of the particles during the growth of the initial mesoporous silica shell. Furthermore, the larger size of solid SiO$_2$ nanoparticles with 40 ± 5 nm diameter was prepared by the sol–gel route (Fig. 8g) [44]. These nanoparticles remained stable in aqueous medium due to the electrostatic repulsion from the negatively charged silanols. Here the adsorption of CTAB was used to func-

![Fig. 6. Normalized photoluminescence spectra of (a) as-prepared CdTe nanocrystals and (b) CdTe@MSNs nanocomposites at the excitation of 450 nm. For each spectrum, the selected CdTe from left to right were green, orange, red and near-infrared quantum dots, respectively. The insets were photographs of aqueous dispersions for the corresponding materials taken under 365 nm UV lamp. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)](image-url)
tionalize the SiO₂ surface affording with positively charge. In Fig. 8h, the typical core/shell structure of the SiO₂@MSNs nanocomposites could be discerned clearly, which contained a solid core and a wormhole-like mesoporous shell. The obtained uniform SiO₂@MSNs nanocomposites were about 70 ± 10 nm in diameter, and the ordered mesoporous silica shell was about 15 nm in thickness. These core/shell nanocomposites were very stable in pH 7.4 phosphate buffer (0.15 M) and could be stored for over a long time at room temperature without observation of any precipitation.

The sizes of all the prepared nanocomposites (MSNs coated CdTe, Fe₃O₄, Au nanoparticles, Au nanorods and SiO₂ nanoparticles) were uniform with a narrow polydispersity index of about 0.1 and a mean hydrodynamic size less than 160 nm, which are within applicable size range for drug and gene delivery[11]. The particle size and suspension stability could play an important role in the next in vitro cellular uptake application.

3.3. The in vitro cell assay of CdTe@MSNs nanocomposites

The toxic nature of semiconductor quantum dots limits their applications in biology and medicine fields, because it had been pointed out that the surface oxidation of the CdTe nanocrystals resulted in a release of surface cadmium atoms, which could be extremely toxic to cells [45]. Therefore, the investigation on cytotoxicity of CdTe@MSNs nanocomposites was necessary for being potentially applied as effective drug carrier for therapeutic treatment of tumors. The in vitro cytotoxicity against 293 normal cells and HeLa cancer cells were investigated by MTT assay, respectively. As shown in Fig. 9, the carrier of CdTe@MSNs nanocomposites showed almost no cytotoxicity to HeLa cells even up to a high concentration of 100 μg/mL. For 293 cells, the CdTe@MSNs had little decrease in cell viability with increasing the concentration from 0.1 to 50 μg/mL, exhibiting a good biocompatibility for the nanocomposites. However, a slight reduction in cell viability of about 80% was kept at the concentration of 100 μg/mL, indicative of a slight degree of cytotoxicity at higher concentrations. Based on the results of above cytotoxicity, we speculated that MSNs coated CdTe core/shell nanocomposites could efficiently improve the stability of CdTe quantum dots, which critically increased the biocompatibility of semiconductor nanocrystals. This model demonstrated the potential of these multifunctional MNSs for application as a fluorescently trackable carrier, which can be loaded with different drugs and simultaneously monitored.

The effective cellular uptake of drug delivery vehicles can enhance the delivery efficiency and achieve the desired therapeutic action. As CdTe quantum dots can be incorporated into the mesoporous silica walls, the resultant CdTe@MSNs nanocomposites with near-infrared fluorescence had the potential to be used simultaneously for optical imaging and drug delivery system. The uptake results of the CdTe@MSNs nanocomposites by RBL (Fig. 10a) and HeLa (Fig. 10b) cells were studied by confocal laser scanning microscopy (CLSM) under in vitro conditions. The images from left to right column showed the bright field, fluorescent and merged images of cancer cells incubated with CdTe@MSNs nanocomposites (100 μg/mL) in cell culture media for 4 h at 37 °C, respectively. From the images of CLSM in Fig. 10a, it could be seen that the RBL cells remained attached on the plate well and maintained their normal morphology after being incubated with the CdTe@MSNs nanocomposites, which implied that the nanocomposites had no cytotoxic effect on the RBL cells. In addition, red fluorescence of CdTe@MSNs was also observed in the cytoplasm of RBL cells. The significant internalization of the nanocomposites was demon-
Stratified by the presence of spot-like fluorescence inside the cytoplasm via endocytosis rather than absorbed on the exterior of cells [46], which was further supported by the overlay of the bright field and fluorescent imaging. Notably, the luminescent CdTe@MSNs could hardly enter into the cell nucleus. There was not much difference in the uptake behaviors for HeLa cells (Fig. 10b), compared with RBL cells, demonstrating that the nanocomposites had no preference toward various cancer cells. In this regard, the above qualitative cell uptake results suggested the feasibility and efficiency of near-infrared CdTe@MSNs nanocomposites as carriers for simultaneous fluorescence cell imaging and anti-cancer drug delivery into cancer cells.

3.4. The capability of nanocomposites as drug delivery system

To investigate the drug loading and release performance of CdTe@MSNs nanocomposites, we selected DOX as a model drug, which has been extensively investigated for sustained and controlled drug release due to its good pharmacological activity, dose-dependent cardiotoxicity and suitable molecule size [47]. The amount of DOX loaded in the nanocomposites was determined by UV spectrometry, reading at 485 nm, resulting in about 22 wt.% for loading content and 94 wt.% for embed efficiency with respect to the starting addition. Employing as drug carriers, CdTe@MSNs nanocomposites revealed a high loading capacity attributing to the larger space for storing DOX molecules [48], provided by the huge pore cavities in the mesoporous shell.

The pH-dependent profiles of DOX release from CdTe@MSNs nanocomposites were conducted. As was evident from the release behaviors shown in Fig. 11, DOX-loaded CdTe@MSNs exhibited significant pH-dependent release trends at pH 5.0, 6.5 and 7.4, having the release amount of approximate 89.7%, 43.1% and 19.1%, respectively. This pH-responsive drug release property was mainly ascribed to the electrostatic interaction mechanism. Mesoporous silica is always negatively charged because of the isoelectric point (pI) at pH 2–3 [49]. As pH elevated, the surface electric potentials were significantly strengthened, which resulted in \( \phi_{0} \) of \(-13.2\), \(-24.1\) and \(-30.8\) mV indexed to pH 5.0, 6.5 and 7.4, respectively. Therefore, in the case of acidic medium (pH 5.0), the electrostatic attraction between the carriers and DOX (a basic compound with pI 8.25) was weakened. In this regard, it was expected that the loaded DOX would be released quickly at pH 5.0 compared with that at neutral pH. Meanwhile, the faster release trend at mildly acidic pH also benefiting from the enhanced hydrophilicity and higher solubility of DOX at lower pH causes increased protonated \(-\text{NH}_2\) groups on DOX molecules [43].

This pH-sensitive releasing behavior was of particular interest in achieving the tumor-targeted DOX delivery with CdTe@MSNs. It was expected that a slight faster release will occur when the CdTe@MSNs nanocomposites reached the extracellular environment of solid tumor tissue where the pH value is more acidic.
Furthermore, an apparent accelerated drug release inside the endosome/lysosome after cellular uptake will happen due to the further decreased pH values (pH 4.5–6.4). The technology for the synthesized CdTe@MSNs nanocomposites will be helpful in traceable delivery and controlled release of therapeutic agents by incorporating drugs into the mesoporous silica pores.

4. Conclusion

The results presented here showed that multiple types of hydrophilic inorganic nanoparticles (CdTe, Fe₃O₄, Au nanoparticles, Au nanorods and larger solid SiO₂ nanoparticles) were directly employed as seeds for the synthesis of core/shell nanocomposites through one-pot method, without requiring other modification procedures. By adsorbing the bilayer of cationic CTAB surfactant onto the colloidal surface, these nanoparticles were transferred from negatively charge to positively charge drastically. Then, a silica precursor TEOS hydrolyzed, condensed and produced a cooperative self-assembly with the bilayer of CTAB overcoated nanoparticles and the free CTAB micelles to form the mesostructure under basic conditions. The relatively high CTAB concentration and proper pH value in the initial reaction system were the key factors for synthesizing uniform, colloidal CdTe@MSNs nanocomposites with obvious core/shell structure. In an optimized recipe CTAB content of 2 mg/mL and a fixed starting pH value of 11.7, uniform sized nanocomposites of 130 nm diameter and 960 m²/g surface area with high CdTe occupancy were prepared. In general, increasing the CTAB concentration reduced the number of the incorporated CdTe nanocrystals inside of MSNs, and correspondingly the fluorescence intensity of nanocomposites could also be regulated effectively. The controlled preparation method of multifunctional silica nanocomposites provided the platform for designing multifunctional MSNs to assess biological effects. In in vitro cell assays, the nanocomposites showed no cytotoxicity to 293 cells at low concentrations and ease of uptake into cancer cells. The anticancer DOX was successfully loaded into the nanocomposites with a high efficiency and the drug release behavior exhibited an apparent pH-response: a low leakage of only 19.1% at pH 7.4 but an enhanced release of 89.7% in 24 h at pH 5.0. Therefore, the synthesized multifunctional nanocomposites comprising various characters of as-reported nanoparticles, along with their potential use as a drug delivery vehicle, are expected to be useful in cancer diagnosis and therapy.

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References


