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DOX-CONJUGATED HIGH-QUALITY AgZnInS QDs FOR REVERSAL OF MULTIDRUG RESISTANCE

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The synthesis of water-soluble quantum dots (QDs) has recently received extensive attention due to noninvasive detection of biological information in living subjects. In this paper, high-quality water-soluble (cadmium-free) quaternary AgZnInS QDs have been successfully synthesized using a green synthetic route. The as-prepared QDs exhibit tunable photoluminescence (PL) emission between 521 and 658 nm. Secondly, multidrug resistance (MDR) is a major impediment to the effective cancer chemotherapy. DOX, a widely used antitumor drug was modified on the surface of the QDs in this study. It, therefore, significantly enhanced the cytotoxicity of DOX to MDR cancer cells as the QDs could bring the DOX to nucleus.

Keywords: AgZnInS QDs; doxorubicin; multidrug resistance (MDR).

1. Introduction

Medical imaging techniques, such as computed tomography (CT), magnetic resonance imaging (MRI), positron emission tomography (PET), ultrasound imaging, and optical imaging, play key roles in disease diagnosis.[1,2] Each imaging technique (or modality) can bring unique information to molecular medicine. Unlike other imaging modalities, optical imaging uses low energy radiation in the visible or near-infrared (NIR) regions of light to assess biological processes. Due to quantum confinement effects, semiconductor quantum dots (QDs, or nanocrystals) exhibit special physical and chemical properties which are greatly distinct from those of the corresponding bulk materials.[3,4] Hence, QDs have wide potential applications as biomedical labels, lightemitting diodes (LEDs), lasers, etc[5,6]. Over the past two decades, great efforts have been put into the synthesis of the highly fluorescent II-VI semiconductor QDs. As a result, a large number of high-quality QDs, such as CdSe and CdTe QDs with high

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fluorescence, conveniently tunable size, and narrow size distribution, have been successfully prepared through an organometallic route or an aqueous-based approach. However, their efficient emission mostly ranged from green to NIR region (500–750 nm), i.e., relatively limited reports on the synthesis of QDs with violet–blue emission between 400 and 500 nm, especially in aqueous media. Blue is one of the tricolors. Hence, violet–blue fluorescence is very important for optoelectronic devices and multicolor biomedical imaging. Over the past years, the development of new fluorescent semiconductor QDs for optical imaging has attracted much attention.[7,8] Compared with the use of organic dyes as imaging probes, QDs are a novel class of materials with unique optical and electronic properties, such as size-tunable PL, high PL quantum yield (QY), sharp and symmetrical fluorescence peak, broad excitation spectrum, large Stokes shift, multicolor fluorescence with a single-wavelength excitation source, and high resistance to photobleaching. Unfortunately, most of the highly luminescent QDs currently used for biomedical imaging are composed of toxic elements (Cd, Hg, Pb, Se, Te, As etc). Several groups' reports have shown the cytotoxicity of QDs is due to the eventual release of toxic components into the cellular environment. This represents a major obstacle to the clinical use of QDs and has motivated the development of new biocompatible QDs based on the use of I-II-III or I-III-VI_2 materials with relatively low toxicity.[9,10] ZnAgInS QD is a I-II-III semiconductor QD, corresponding to a 600 nm emission wavelength, and does not contain highly toxic heavy metals. Therefore, this material might be a promising candidate for optical imaging, which offers the opportunity to develop semiconductor QDs without the toxicity limitations encountered by II-VI QDs, especially at low concentrations. Recently, several colloidal chemistry approaches have been employed to synthesize ZnAgInS or other I-II-III QDs. In this paper, a direct aqueous synthetic approach was used to prepare AgZnInS (ZAIS) QDs, this method is simple, cost-effective, environmentally friendly, and allows for biocompatibility.

Multidrug resistance (MDR) is an important reason for the failure of antiinfective therapy and chemotherapy. In 2010, "superbugs" appeared which are also multi-drug resistant. MDR is mainly caused by variations in lactamase and overexpression of membrane efflux protein, which can pump out anticancer drugs, such as P-glycoprotein (P-gp) overexpression. An important membrane transporter involved in MDR is P-gp, which is overexpressed in the plasma membrane of MDR tumor cells and is capable of effluxing various anticancer drugs (e.g., doxorubicin and paclitaxel) out of the cells. Nanoscale vehicles have unique physical and biological properties. They can be used as drug carriers for targeting tumors and improving the anticancer efficacy of drugs and may provide opportunities for overcoming MDR. Many nano-based drug delivery systems have been used for the inhibition of drug efflux mediated by P-gp.[11,12] Recently, it has been demonstrated that nanoparticles showing responsibility to intracellular stimulus are capable of delivering chemotherapeutic drugs to overcome MDR. Bae's group has carried out intensive studies on folic acid-conjugated polymer micelles, which are sensitive to early endosomal pH. It has been suggested that MDR cancer cells may have limited capacity to defend themselves against cytotoxic chemicals.[13] In this sense, realizing a sufficiently high intracellular level of cytotoxic chemicals using an optimized delivery system might represent a novel tactic in overcoming the MDR of cancer cells, while the intracellular accumulation of drug and the intracellular release of drug molecules from the carrier could be the most important barriers for nanoscale carriers in overcoming MDR.

2. Experimental Section

Chemicals: Silver(I) nitrate (99.9%), indium (III) acetic (99.99%), zinc (II) acetic (99%), sodium hydroxide, sodium citrate (99%), sodium sullfide, polyacrylic acid sodium salt (PAA, MW 1200), mercaptoacetic acid (MAA, 98%), and citric acid (CA, 99%) were purchased from Sigma-Aldrich. Glutathione (GSH, 98%) was purchased from International Laboratory, USA. Double distilled water was prepared using GFLM-2302 water distiller. RPMI 1640 medium, calf serum, penicillin, streptomycin, trypsin, and EDTA were purchased from commercial sources. The water used in all experiments had a resistivity higher than 18.2 $M\Omega \cdot cm$. All chemicals were used without further purification. Human hepatoma cell line (Bel-7402), human lung carcinoma cell line (A549) were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA).

2.1. *Synthesis of ZnAgInS (ZAIS) QDs*

0.5 mL of Zn^{2+} solution (0.05 M), 0.1 mL of Ag^{-} solution (0.05 M), 1 mL of In^{3+} solution (0.05 M), and 5 mL of GSH and PAA solution were loaded in a three-neck flask. Then, the pH of the solution was adjusted to 8 with 0.8 M of NaOH solution under stirring. Subsequently, a certain amount of freshly prepared Na_2S solution was quickly injected into the above reaction flask. Here, the total volume of the reaction solution was set to 50 mL. Finally, the resulting mixture was heated to $\sim$100°C under open-air atmosphere with condenser, and refluxed for 4 h to promote the growth of the quaternary ZAIS QDs.

2.2. *ZAIS QDs for cell imaging*

A549 cells were cultured in RPMI 1640 medium with 10% (v/v) calf serum at 37°C (5% CO_2) and grown in a 24-well plate. After seeding for 24 h, the medium was aspirated, and 2 mM ZAIS QDs was added to the wells. After incubation for 2 h, the medium was aspirated and the cells were washed with 1 × PBS three times for 5 min each. Then, the cells were in combination with Hoechst solution for 20 min and were washed with 1 × PBS three times for 5 min each. The fluorescence images of the cells were obtained with an Olympus Fluoview 300 confocal laser scanning system with 488 nm argon laser excitation.

2.3. *QDs modified with DOX*

The water-soluble drug, doxorubicin (Dox), was efficiently packaged into ZAIS *via* an electrostatic mechanism. Five μg/ml DOX was added into as-prepared QDs, standing for 24 h. Next, DOX-ZAIS QDs compound was precipitated with ethanol to remove excess unreacted precursors and redispersed in water. A 754-PC UV-Vis spectrophotometer (JH 754PC, Shanghai, China) was employed for the measurement of UV-Vis spectrum of as-prepared DOX-QDs compound.

2.4. *Characterization*

A S2000 eight-channel optical fiber spectrophotometer (Ocean Optics Corporation, America) and a broadband light source (X-Cite Series 120Q, Lumen Dynamics Group Inc., Canada) were utilized for the detection of PL emission spectrum. A 754-PC UV-Vis spectrophotometer (JH 754PC, Shanghai, China) was employed for the measurement of UV-Vis absorption spectrum. PL QY of QDs in water was calculated by comparing their integrated emission to that of R6G in ethanol (QY = 95%).[14] TEM images were taken on a JEOL JEM-2100 transmission electron microscope with an acceleration voltage of 200 kV.

3. Results and Discussion

3.1. *Synthesis of ZnAgInS (ZAIS) QDs*

A direct aqueous synthetic approach was used to prepare ZnAgInS (ZAIS) QDs. In contrast to the conventional organic phase synthetic route this method is simple, cost-effective, environmentally friendly, and allows for biocompatibility. In our aqueous synthesis of ZAIS QDs, quaternary ZnAgInS (ZAIS) QDs were initially produced by the reaction of silver nitrate, zinc acetate, indium acetate with polycarboxylate (i.e., PAA) and thiol-functionalized (i.e., MAA) ligands in the presence of sodium sulfide at 50°C. Subsequently, sodium sulfide was added quickly, followed by intense stirring. The solution turned to yellow quickly as sodium sulfide disintegrated. Quaternary ZnAgInS (ZAIS) QDs were synthesized at 103°C. In this paper, we focus on the effect of the thiol-functionalized (i.e., MAA) ligands on the quaternary ZnAgInS (ZAIS) QDs fluorescence properties. Combination of thiol-functionalized ligands as stabilizing and reactivity-controlling ligands was used to prepare AgZnInS (ZAIS) QDs. We observed that different combinations of thiol-functionalized ligands and different molar ratios of ligands have influence on the ZnAgInS (ZAIS) QDs fluorescence properties. Using GSH and MAA as stabilizing and reactivity-controlling ligands shows best results rather than a combination of GSH and CA, and GSH and PAA (Fig. 1(a)). As one can see in Figs. 1(b) and 1(c), a 1:1 molar ratio of GSH:MAA and a 1:3 molar ratio of CSH:PAA yielded optimum results (Fig. 1). The thiol-functionalized MAA ligand is a Lewis base that binds preferentially to Ag^{+}, which is a Lewis acid. On the other hand, a large amount of PAA relative to that of GSH (3:1 ratio) is found to be necessary. PAA, a polymer with multiple carboxylate groups, is a hard Lewis base that preferentially reacts with hard Lewis acids like In 3^{+}. Moreover,

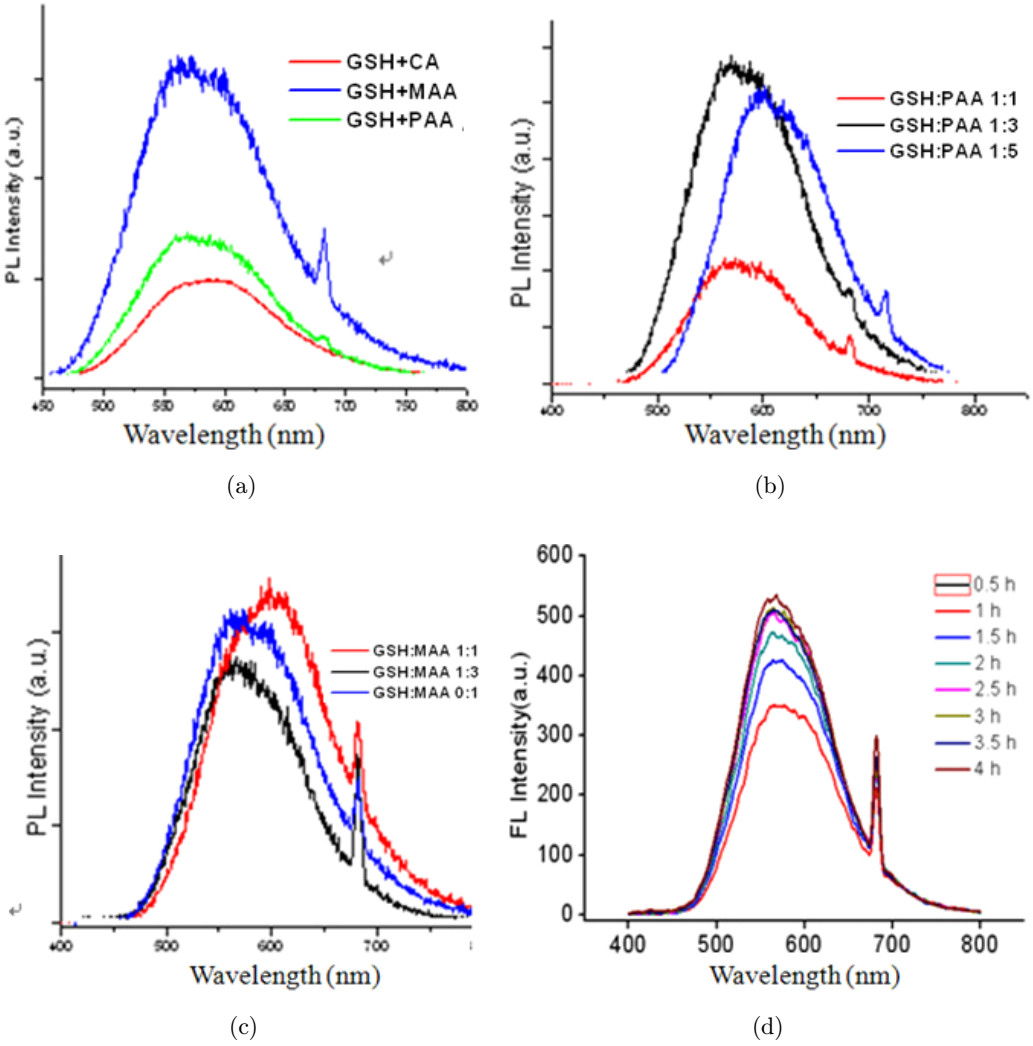

Fig. 1. PL spectra of the prepared water-soluble ZAIS QDs.

its polycarboxylate functionality allows for good colloidal stability of the QDs in water. Thus, apart from regulating the reactivity of the indium precursor, its presence in the reaction mixture also provides good water-dispersibility to the generated QDs. We find that when decreasing the MAA concentration, the emission peak red shifts gradually from 521 to 658 nm. This is because the growth of ZAIS QDs is primarily controlled by the competition between the S^{2+} and thiol groups of ligand for linking to the cations on the growing particle surface. The lower the concentration of ligand, the lower is the density of ligand molecules existing on the surface of the growing particle, resulting in an increase in the size of as-prepared QDs. These results indicate that it is possible to conveniently tune the fluorescence color of ZAIS QDs by controlling the ligand concentration, besides by changing the thiol-functionalized ligands.

Next, the PL and absorption spectra of the as-prepared water-soluble ZAIS QDs were further measured. The results show that highly fluorescent water-soluble ZAIS QDs (PL QY, $\sim$25%) could be obtained by using GSH:MAA 1:1 as surface-modifying agent (with GSH:PAA 1:3 PL QY, $\sim$8%), whereas with GSH:CA the resulting aqueous ZAIS QDs showed very weak fluorescence signal.

3.2. *Morphology characterization*

Transmission electron microscopy (TEM) was used to investigate the morphology of ZAIS QDs. Figure 2 shows TEM image of the prepared QDs, indicating these nanocrystals were close to spherical

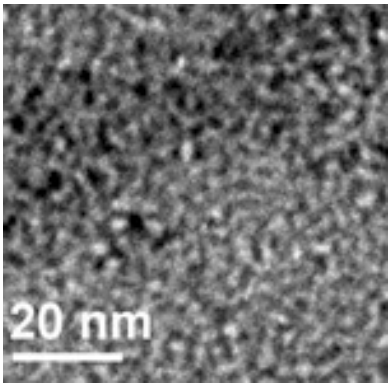

Fig. 2. TEM image of ZAIS QDs.

with excellent polydispersity and average size of about 2.5 nm.

3.3. *ZAIS QDs for cell imaging*

As described above, using GSH and MAA as stabilizers, water-soluble ZAIS QDs with fluorescence emission in the yellow–red spectral range have been synthesized. Next, we explored preliminarily the fluorescence imaging of ZAIS QDs to demonstrate its potential application in cell biology. In brief, A549 cells were incubated with 2 mM of ZAIS QDs for 4 h, and hoechst for 20 min. The brightly-filled fluorescence images of cells are displayed in Fig. 3. These photomicrographic images reveal that the size of ZAIS QDs is very small and easy to be endocytosed by the cells. As a result, the cells nucleus labeled by as-prepared QDs present intense fluorescence. These further confirm that the resulting high-quality ZAIS QDs should have wide potential applications in biolabeling and cell imaging.

3.4. *Conjunct with DOX*

MDR is a major obstacle to successful cancer chemotherapy. Although cancer cells can be obtained through a variety of molecular mechanisms of MDR, P-gp is an important membrane transporter

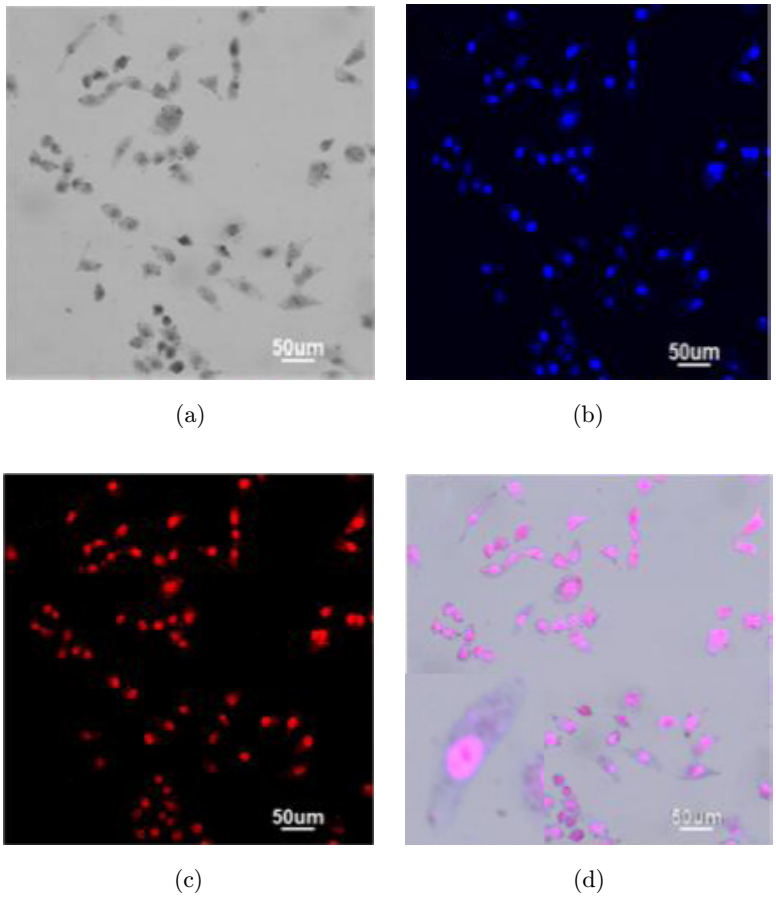

Fig. 3. Optical microscopy of live A549 cells incubated with QDs (a) differential interference contrast (DIC), (b) hoechst, (c) QDs, (d) the merged image of the fluorescence images and DICimage. λex = 488 nm. (inset: cell imaging with higher magnification.)

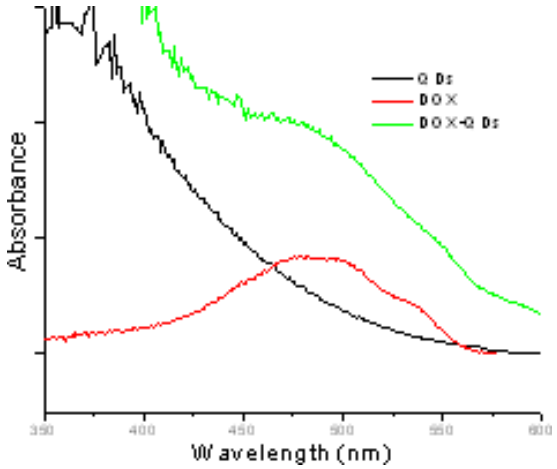

Fig. 4. UV-Vis spectrum of as-prepared DOX-QDs compound.

involved in MDR. MDR tumor cells overexpress P-glycoprote on the cell membrane, which can efflux various anticancer drugs out of cells (such as doxorubicin and paclitaxel). Intracellular release of the drug from the carrier is probably the most important obstacle to overcome MDR. In this sense, the use of the delivery system to achieve a sufficiently high intracellular levels of cytotoxic substances may represent a new tactics to overcome MDR cancer cells. Figure 4 shows the ultraviolet absorption of as-prepared DOX-QDs compound, DOX was successfully modified on the surface of QDs. As the ZAIS QDs can easily reach the cells' nucleus, maybe the DOX, modified on the surfaces of QDs, can be carried to the cells' nucleus, sites of action of DOX.[13,15,16]

4. Conclusion

In this paper, high-quality water-soluble (cadmium-free) quaternary AgZnInS QDs have been successfully synthesized using a green synthetic route. DOX was modified on the surface of the QDs in this study. It may significantly enhance the cytotoxicity of DOX to MDR cancer cells as the QDs could bring the DOX to nucleus.

Acknowledgments

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