Cytotoxicity and cellular imaging of quantum dots protected by polyelectrolyte

Hai-Yan Hu\textsuperscript{a,b,1}, Xing-Ru Dou\textsuperscript{b,1}, Zong-Lin Jiang\textsuperscript{a,*}, Jian-Hua Tang\textsuperscript{b}, Lian Xie\textsuperscript{b}, Hong-Ping Xie\textsuperscript{b,*}

\textsuperscript{a}College of Chemistry and Chemical Engineering, China West Normal University, Nanchong 637002, P.R. China
\textsuperscript{b}College of Pharmaceutical Science, Soochow University, Suzhou 215123, P.R. China

Received 11 November 2011; accepted 13 February 2012
Available online 21 February 2012

Abstract The nanocomposites of poly-diallyldimethylammonium chloride (PDADMAC) and CdTe quantum dots (QDs) (i.e. QDs-PDADMAC nanocomposites) have been prepared based on electrostatic interaction and their fluorescence stability in aqueous solution has been investigated. MTT method (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide method) was used to study their cytotoxicity and A549 lung cancer cell as a model cell was also used to evaluate their cellular imaging. It was shown that the fluorescence stability of QDs-PDADMAC nanocomposites was much better than that of bare QDs both in aqueous solution and cell. Meanwhile, QDs-PDADMAC nanocomposites display very low cytotoxicity in the low concentrations and better staining ability compared with QDs. QDs-PDADMAC nanocomposites will have great advantage on the cell analysis detection and imaging.

1. Introduction

II–VI semiconductor nanocrystals with a diameter of 2–10 nm, also known as quantum dots (QDs), have unique optical properties \cite{1–4}, including: (1) the emission wavelength could be changed by adjusting the size of QDs and changing materials, that is “tune capability”, and the QDs with different fluorescence spectrum characteristic could be prepared; (2) QDs have strong fluorescence emissive power; (3) QDs have wide excitation spectrum with same range of wavelength, and then different QDs could be excited by same wavelength; (4) QDs have better fluorescence stability than fluorescence dye. Owing to these advantages, QDs have attracted considerable attention in the field of biology and medicine over the past two decades \cite{4–7}. Certainly, cellular imaging is one of important usages in biology and medicine, and it is an important method...
of cellular analysis, especially analysis of biological procession in cell. However, two important properties could influence on the usage of QD’s in cellular imaging, that is, cytotoxicity and fluorescence stability, especially fluorescence stability in cell.

More and more people have paid attention to the cytotoxicity of QDs and their toxicity for organism. Up to now, various studies have shown that the toxicity of QDs is affected by many factors, including physico-chemical property of the particles themselves (such as particle size, stability, dispersion, surface charge, surface modification groups, oxidation state), the concentration of quantum dots, the receptor cells (in vivo) coming from different species, and culture (exposure) time [8–11].

There are two kinds of QDs. One is oil-soluble and the other one is water-soluble. It’s necessary for oil-soluble QDs to be modified on their surface, which could make them react in aqueous solution easily as well as cytols. However, such surface modification would let the fluorescence stability of QDs greatly reduce. For the water-soluble QDs, ligand on the surface modification would let the fluorescence stability of QDs in aqueous solution easily as well as cytosol. However, such kind of QDs is used in cellular imaging, the fluorescence imaging results will not be satisfactory.

For water-soluble QDs, CdTe ones are one of the most important QDs focused [9,12–15]. The CdTe QDs were used as one model QDs studied in this paper, and then the after “QDs” and “QDs-PDADMAC nanocomposites” should denoted CdTe QDs and the nanocomposites of CdTe QDs and poly-diallyldimethylammonium chloride (PDADMAC), respectively. In this study, QDs-PDADMAC nanocomposites in aqueous solution have been prepared by facile one-step electrostatic interaction and cytotoxicity and cellular imaging have been studied to compare QDs-PDADMAC nanocomposites with QDs without protection of polyelectrolyte. The results showed that QDs with protection of polyelectrolyte have better fluorescence stability than bare QDs. What’s more, QDs-PDADMAC nanocomposites have advantage on cytotoxicity and cellular imaging as well, and show cell analysis detection potential.

2. Experimental

2.1. Materials

Poly-diallyldimethylammonium chloride (PDADMAC 35% in water, MW=10152) was purchased from Aldrich. Tellurium powder (99.99%), NaBH₄ (95%) and CdCl₂·2.5 H₂O (99.99%) thioglycolic acid (TGA, ≥98%) were all purchased from SCRC (Sinopharm Chemical Reagent Co., Ltd). A549 lung cancer cell was provided by College of Pharmaceutical Science, Soochow University. Modified RPMI-1640 medium was purchased from Thermo Fisher Biochemical Products (Beijing) Co., Ltd. 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl-tetrazolium bromide (MTT) was bought from Amresco. Other chemicals were of analytical grade or better. Milli-Q water at 18.2 MΩ cm was used throughout.

2.2. Preparation of QDs-PDADMAC nanocomposites

The synthesis of CdTe QDs was according to Ref. [15]. 1 mL non-defective CdTe QDs and 300 μL PDADMAC with the concentration of 30 mg/mL were mixed in a small ampoule and stirred for 20-30 min. Then the mixture was centrifuged by adding the mixture of absolute alcohol and acetone (1:1, v/v) in order to remove excessive TGA and PDADMAC, and then the precipitation was redissolved in 1 mL H₂O. Finally the QDs-PDADMAC stock solution was obtained, which was stored in dark environment.

2.3. Cytotoxicity assay by MTT method

In this paper, MTT method was used to evaluate cytotoxicity of QDs using A549 lung cancer cell as model cell. A549 lung cancer cell with 7 × 10⁴ cells/mL was seeded into 96-well plates and maintained 24 h in 200 μL of the cell culture medium (Modified RPMI-1640 medium). These cells were divided into three groups which were named QDs-PDADMAC, QDs and the negative control group. Then the same volume of QDs-PDADMAC and QDs solutions was added to each well of QDs-PDADMAC and QDs groups, respectively. The cell culture medium, whose volume was the same with the QDs-PDADMAC or QDs solution, was added to the negative control group. At the same time, all wells of the blank control group in the 96-well plates contained the cell culture medium only. They were all incubated for 24 h at 37 °C. Then 20 μL of the freshly prepared MTT (1 mg/mL in PBS buffer with pH 7.4) was added to each well and incubated for 4 h at 37 °C. After removing the solution, the cells were lysed by adding 200 μL methyl sulfoxide. The 96-well plate was gently shaken for 10 min, and then the absorbance of the produced purple formazan at 570 nm was monitored using a Bio-Tek ELX800 plate reader.

2.4. Cellular imaging assay

A549 lung cancer cell was seeded into 6-well plates and maintained in 2mL Modified RPMI-1640 medium for 24h. These cells were divided into three groups which were named QDs-PDADMAC, QDs and the control group. Then, 1 μL QDs-PDADMAC and QDs solutions with the same fluorescence intensity were added to each well of QDs-PDADMAC and QDs groups, respectively. The same volume of culture medium was added to the control group. After incubated for 24 h at 37 °C, all the wells of three groups were washed twice with PBS buffer (pH 7.4). The glass slides as the fluorescence images were prepared using the cover glass of the 6-well plates. Then, the fluorescence images were recorded with a Nikon CiSi confocal laser scanning fluorescence microscope with 405 nm excitation.

3. Results and discussion

3.1. Transmission electron microscopy morphology and fluorescence stability of QDs-PDADMAC nanocomposites

The transmission electron microscopy (TEM) photograph of QDs-PDADMAC nanocomposites is shown in Fig. 1. It presented that this kind of the nanocomposites showed curled-shaped band-like morphology with unequal length. The width of the band was 4–8 nm. This special morphology of QDs-PDADMAC nanocomposites may result from the relative scales and the opposite charges between CdTe QDs and the polyelectrolyte.
PDADMAC. Since the surface electric charge of CdTe QDs is negative one and the cationic polyelectrolyte PDADMAC has rich positive charge, the electrostatic interaction could be caused each other. At the same time, CdTe QDs are a kind of the nanoparticles with the small diameter (about 1–2 nm), and PDADMAC is the polyelectrolyte with long-chain (about 10000 of molecular weight). Based on the electrostatic interaction, layer-by-layer self-assembling may be caused between long-chain cationic polyelectrolyte and small size QDs with negative charge, which results in curly-shaped band-like morphology of the QDs-PDADMAC nanocomposites with unequal length.

As the formation of this special morphology of QDs-PDADMAC nanocomposites, TGA was wrapped in the band and was not easily drop out from the surface of QDs, which ensured the fluorescence stability of QDs (Fig. 2). As shown in Fig. 2, with the extension of storage time, the fluorescence intensity of QDs-PDADMAC nanocomposites did not decrease, and there’s a trend of increase. Compared with QDs-PDADMAC nanocomposites, fluorescence intensity of QDs gradually decreased, especially when the sixth day, QDs hardly showed effective fluorescence intensity. Therefore, bare QDs which are unprotected are difficult for practical applications, and they are impossible to have good performance on cellular imaging.

3.2. Cytotoxicity of nanoparticles

In this paper, the as-prepared QDs-PDADMAC nanocomposites have good fluorescence stability. At the same time, in order to evaluate the fluorescence stability in cells, cellular imaging of QDs-PDADMAC nanocomposites has been studied. Before cellular imaging assay, cytotoxicity was tested. A549 lung cancer cell was used as model cell in cytotoxicity and cellular imaging assays.

The cytotoxicity of as-prepared nanoparticles, which was used to evaluate their biocompatibility, was studied using A549 lung cancer cell as a model cell by the MTT method. As MTT is able to enter the viable cells and produce a chemical component with a characteristic absorption at 570 nm and the dead cells do not have this character, the absorbance at 570 nm is dependent on the degree of activation of the cells. Then, the cell viability was expressed as:

\[
\text{Cell viability} = \frac{(OD_x - OD_0)}{(OD_s - OD_0)} \times 100\%
\]

Here ODx, OD0 and ODs represent the absorption of test samples (i.e. the samples in the QDs-PDADMAC nanocomposites or QDs group), blank control ones and negative control ones at 570 nm, respectively. As shown in Fig. 3, with the amount of fluorescent nanoparticles increases, the cell viability decreased gradually, indicating that the toxicity increased. Compared with QDs, QDs-PDADMAC nanocomposites had lower cytotoxicity at low concentration, while at high concentration the cytotoxicity of QDs-PDADMAC nanocomposites was obviously higher than that of QDs. It may be related with PDADMAC which is strong electrolyte. High concentration of QDs-PDADMAC in cell culture medium, equivalently to the strong electrolyte in culture medium, could reduce cell viability even lead to apoptosis because of hypertonic effect of PDADMAC which could lead to cell dehydration. With high intensity...
and good fluorescence stability, QDs-PDADMAC nanocomposites could be used in cellular imaging at low concentration and the nanocomposites have low cytotoxicity at low concentration as well. Therefore, the QDs-PDADMAC nanocomposites will be well applied in cellular imaging, which will have great biological significance for cell analysis detection.

3.3. Cellular imaging of nanocomposites

The as-prepared QDs-PDADMAC nanocomposites were studied to evaluate its application to biological imaging using A549 lung cancer cell as a model cell. The image (Fig. 4) showed that the staining effect of QDs-PDADMAC nanocomposites was much better than that of QDs. In QDs group, the cells were almost not stained, similarity to the control group. However, the cells of QDs-PDADMAC nanocomposites group could be observed clearly. The result could be explained by our above discussion. The ligand of QDs was easy to drop during the cell culture, resulting in the fluorescence being quenched. The fluorescence stability of QDs assembled by PDADMAC has been improved and did not suffer from adverse effects, which led to good staining effect. Therefore, compared with the bare QDs, QDs-PDADMAC nanocomposites were more suitable for cellular imaging.

4. Conclusion

In this paper, electrostatic self-assembly technology was used to prepare the CdTe nanocomposites that were coated with cationic polyelectrolyte PDADMAC and the nanocomposites showed curly-shaped band-like morphology with 4-8 nm width. With the protection of PDADMAC, the fluorescence stability of QDs-PDADMAC nanocomposites obviously enhanced, and this enhancement effect also reflected in cells. What’s more, the nanocomposites have low cytotoxicity at low concentration, and in this condition QDs-PDADMAC nanocomposites have better cellular imaging effect than bared QDs. Therefore, it implied the as-prepared QDs-PDADMAC nanocomposites in this paper could be applied on intracellular fluorescence detection such as cellular imaging, which indicated that the nanocomposites showed potential on cell detection.

Acknowledgments

This research is supported by the grant from the National Natural Science Foundation of China (Grant No. 81001686) and by the Project Funded by the Priority Academic Program Development of Jiangsu Higher Education Institutions of China.

References

[1] X. Gao, Y. Cui, R.M. Levenson, et al., In vivo cancer targeting and imaging with semiconductor quantum dots, Nat. Biotechnol. 22 (2004) 969–976.
[2] X. Michalet, F.F. Pinaud, L.A. Bentolila, et al., Quantum dots for live cells, in vivo imaging, and diagnostics, Science 307 (2005) 538–544.
[3] J.M. Klostranec, W.C.W. Chan, Quantum dots in biological and biomedical research: recent progress and present challenges, Adv. Mater. 18 (2006) 1953–1964.
[4] M.J. Bruchez, M. Moronne, P. Gin, et al., Semiconductor nanocrystals as fluorescent biological labels, Science 281 (1998) 2013–2016.
[5] W.C. Chan, S. Nie, Quantum dot bioconjugates for ultrasensitive nonisotopic detection, Science 281 (1998) 2016–2018.
[6] K. Manzoor, S. Johny, D. Thomas, et al., Bioconjugated luminescent quantum dots of doped ZnS: a cytotoxic-free system for targeted cancer imaging, Nanotechnology 20 (2009) 65102–65115.
[7] K.T. Yong, Mn-doped near-infrared quantum dots as multimodal targeted probes for pancreatic cancer imaging, Nanotechnology 20 (2009) 15102–15112.

[8] A. Shiohara, A. Hoshino, K. Hanaki, et al., On the cytotoxicity caused by quantum dots, Microbiol. Immunol. 48 (2004) 669–675.

[9] J. Lovric, H.S. Bazzi, Y. Cui, et al., Differences in subcellular distribution and toxicity of green and red emitting CdTe quantum dots, J. Mol. Med. 83 (2005) 377–385.

[10] A. Hoshino, K. Fujioka, T. Oku, et al., Physicochemical properties and cellular toxicity of nanocrystal quantum dots depend on their surface modification, Nano Lett. 4 (2004) 2163–2169.

[11] F.Q. Chen, D. Gerion, Fluorescent CdSe/ZnS nanocrystalpeptide conjugates for long term, nontoxic imaging and nuclear targetting in living cells, Nano Lett. 4 (2004) 1827–1832.

[12] J. Ma, J.Y. Chen, J. Guo, et al., Photostability of thiol-capped CdTe quantum dots in living cells: The effect of photo-oxidation, Nanotechnology 17 (2006) 2083–2089.

[13] J. Li, L. Wang, K. Zhao, et al., Preparation of CdTe nanocrystals and CdTe/SiO₂ nanocomposites in glycol, Colloids Surf. A 257–258 (2005) 329–332.

[14] L.H. Jing, C.H. Yang, R.R. Qiao, et al., Highly fluorescent CdTe@SiO₂ particles prepared via reverse microemulsion method, Chem. Mater. 22 (2010) 420–427.

[15] M. Cao, M.G. Liu, C. Cao, et al., A simple fluorescence quenching method for berberine determination using water-soluble CdTe quantum dots as probes, Spectrochim. Acta. Part A 75 (2010) 1043–1046.