CdSe/ZnS-labeled carboxymethyl chitosan as a bioprobe for live cell imaging†

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Received (in Cambridge, UK) 11th July 2005, Accepted 14th September 2005
First published as an Advance Article on the web 6th October 2005
DOI: 10.1039/b509781a

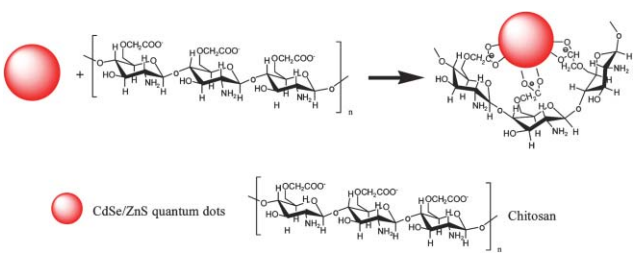
A simple and convenient method for the construction of CdSe/ZnS-labeled polysaccharides as bioprobes were developed, which are highly biocompatible and photostable, and have been proven to be suitable for live cell imaging.

Quantum dots (QDs) have attracted a great deal of attention over the past two decades because of their unique photochemical and photophysical properties. The pioneering work carried out by Alivisatos[1] and Nie[2] in changing QDs from hydrophobic to water-soluble and conjugating QDs with biomolecules has laid a solid foundation for the biological application of QDs. Hydrophobic QDs could be made water-soluble by several methods, most of which rely on the surface-exchange of hydrophobic surfactant molecules for hydrophilic ligands. The most frequently used anchoring groups reactive to the surface of QDs are thiol (–SH) functionalities,[2,4a,b] but other molecules without thiol functionalities[3] were also used, such as 4-substituted pyridine, serotonin, oligomeric phosphine, poly(dimethylaminoethyl methacrylate). If used for surface-exchange, chelating ligands[3c,4] can passivate the surface of QDs more effectively. Besides, conjugation of QDs with a variety of biomolecules, such as DNA, proteins, and small biomolecules, *etc.* has also been well established.[5] However, only one reference reported on the construction of a quantum dot conjugated sugar ball for cellular uptake using four alkyl chain substituted glycocluster amphiphiles and the investigation of the size effects of endocytosis.[6] We report here a simple and convenient method for construction of CdSe/ZnS-labeled polysaccharides as bioprobes and the preliminary results on the uptake of the bioprobes by yeast cells.

Chitin, a naturally abundant amino polysaccharide, and the supporting material of crustaceans and insects, *etc.*, is well known to consist of 2-acetamido-2-deoxy-β-D-glucose, which is quite noteworthy as a new functional material of high potential in various fields.[7] Chitosan and carboxymethyl chitosan (CMC)‡ are water-soluble chitin-like compounds, which can be used to modify QDs *via* chelation of amido and carboxyl groups with heavy metal ions such as phytochelatin-related peptides.[4c] In the present work, polysaccharides were labeled with CdSe/ZnS[8] simply by grinding to produce CdSe/ZnS-labeled polysaccharides. The resultant bioprobes are very stable, water-soluble, dispersive, and narrowly distributed in size, which might be of great potential for the investigation of the biofunctions of polysaccharides.

Tri-*n*-octylphosphine oxide (TOPO)-protected CdSe/ZnS QDs were washed three times with hexane (dissolving) coupled with ethanol (precipitating) to remove TOPO. The freshly washed QDs were dissolved in hexane, then transferred into an agate mortar containing polysaccharide powder, and subsequently the mixture of QDs and the polysaccharide was ground until the hexane completely evaporated. Then additional hexane was added and the mixture was again ground. The grinding process was repeated three times, and the product was dissolved in ultrapure water, thus CdSe/ZnS-labeled polysaccharides were obtained. This simple and facile method is suitable not only for water-solubilization of QDs but also for labeling polysaccharides with QDs.

The possible route for the construction of CdSe/ZnS-labeled polysaccarides, with CMC as an example, is demonstrated in Scheme 1 by chelation[9] based on the results obtained by Fourier transform infrared (FTIR) and X-ray photoelectron spectroscopy (XPS). Bands at 1619.0 and 1415.4 cm^{-1} of CMC assigned to asymmetric and symmetric stretching vibrations of the carboxyl group shifted to 1621.3 and 1417.2 cm^{-1} after labeling with CdSe/ZnS, indicating the coordination of a carboxyl group of CMC with the metal ions on the surface of CdSe/ZnS.[9b] To further confirm the coordination, XPS was used to prove the coordination between the amino group of CMC and CdSe/ZnS. It is well-known that the binding energy of N1s will shift to a low value when nitrogen is bound to metal ions, due to a transfer of electrons



Scheme 1 CdSe/ZnS-labeled carboxymethyl chitosan.

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† Electronic supplementary information (ESI) available: FTIR spectra of carboxymethyl chitosan and CdSe/ZnS-labeled carboxymethyl chitosan; XPS spectra of N1s of CdSe/ZnS-labeled carboxymethyl chitosan and carboxymethyl chitosan; cytotoxicity studies of CdSe/ZnS-labeled carboxymethyl chitosan; synthesis of CdSe/ZnS; preparation of carboxymethyl chitosan. See DOI: 10.1039/b509781a

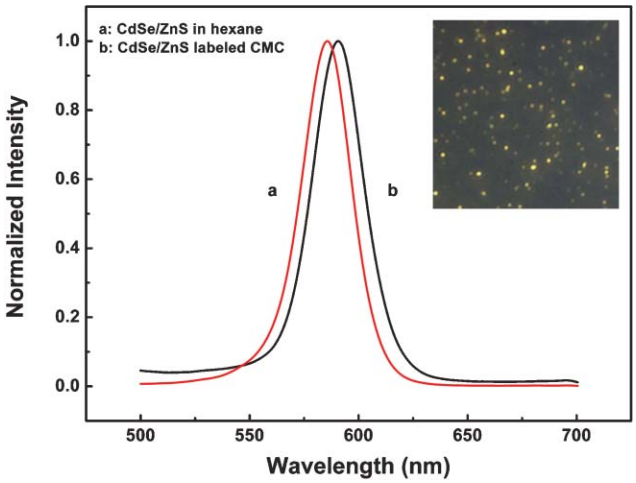

Fig. 1 Fluorescence spectra of CdSe/ZnS in hexane and CdSe/ZnS-labeled CMC in water (top right corner: fluorescence image of CdSe/ZnS-labeled CMC in water).

from nitrogen to the metal ions.[10] The peak positions of N1s of CdSe/ZnS-labeled CMC and CMC are centred at 399.8 eV and 400.5 eV, respectively. The 0.7 eV shift of N1s indicates that the amino group in CMC is coordinated to the metal ions on the surface of CdSe/ZnS.

Water-soluble CdSe/ZnS-labeled CMC has desirable dispersibility, uniformity and good fluorescence proterties characterized by fluorescence spectroscopy, fluorescence microscopy (Fig. 1), and transmission electron microscopy (TEM) (Fig. 2). After grinding, the fluorescence peak position of the QDs red-shifted by about 5 nm. The water-soluble QDs have strong fluorescence and good dispersivity, the fluorescence from a single quantum dot could be observed by fluorescence microscopy (Fig. 1). The particle size of the water-soluble QDs was about 5 nm (Fig. 2), which has desirable dispersivity and uniformity.

When incubated with live yeast cells, CdSe/ZnS-labeled CMC could be found in the interior of yeast cells (Fig. 3). But for the control experiments with CdSe/ZnS-labeled mercaptoacetic acid no uptake was observed. According to the literature,[11] CdSe/ZnS is incompletely biocompatible because of the cytotoxicity of the water-soluble CdSe/ZnS, which might be caused by Cd^{2+} release

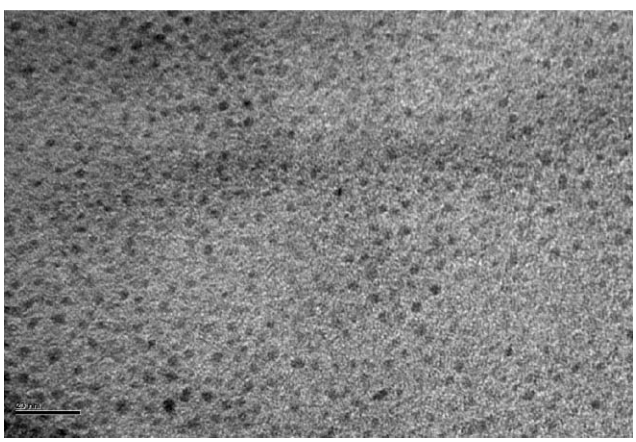

Fig. 2 TEM image of CdSe/ZnS-labeled CMC (scale bar 20 nm).

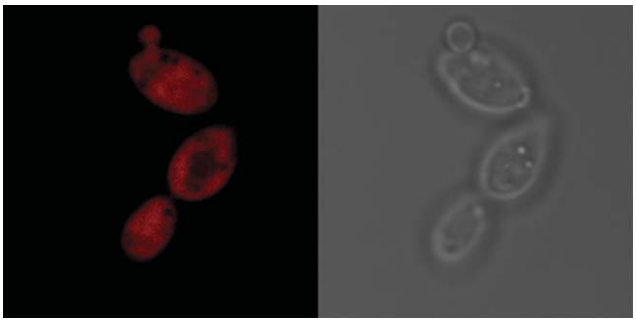

Fig. 3 Confocal images of yeast cells after incubation with CdSe/ZnS-labeled CMC (Left: fluorescence, right: bright field).

and the toxicity of the surface modifier. Hoshino[11*a*] and Derfus[11*b*] envision that cytotoxicity might be prevented by modulation of the surface chemistry of CdSe/ZnS. For this purpose surface modifiers to restrain Cd^{2+} release must be cytocompatible. By staining yeast cells with methylene blue, it has been found that no death of yeast cells resulting from CdSe/ZnS-labeled CMC occurred, however, CdSe/ZnS-labeled mercaptoacetic acid made many yeast cells die (see ESI, Fig. SI-3†). These results suggest that the CdSe/ZnS-labeled CMC scarcely has cytotoxicity, but CdSe/ZnS-labeled mercaptoacetic acid is cytotoxic. The results from colony counting experiments and cell concentration (biomass) determination also confirm that the CdSe/ZnS-labeled CMC is scarcely cytotoxic, and CdSe/ZnS-labeled mercaptoacetic acid is cytotoxic (see ESI†). Because CMC is biocompatible and its chelating groups of carboxyl and amido can coordinate with Cd^{2+} ion to prohibit its release, the CdSe/ZnS-labeled CMC scarcely has cytotoxicity. However, mercaptoacetic acid is not biocompatible, and cannot effectively inhibit Cd^{2+} release. Therefore, CdSe/ZnS-labeled mercaptoacetic acid has cytotoxicity.

There are many ways of intracellular delivery of QDs for live cell labeling and organelle tracking,[12] such as biochemical methods (translocation peptides, cationic liposomes, dendrimers) and physical methods (electroporation and microinjection). As is known, chitin is one of the components of yeast cell wall. The process for the CdSe/ZnS-labeled CMC to enter yeast cells through their wall and membrane might be endocytosis, where polysaccharide CMC like translocation peptides[2] can facilitate the delivery of QDs into yeast cells, which should have been difficult for intact yeast cells.

In principle, QDs can be utilized to track both whole cells and intracellular processes. However, in order to fully exploit the potential of intracellular QDs labeling, well-dispersed QDs are imperative.[12] By confocal microscopy, CdSe/ZnS-labeled CMC was found in the cytoplasm of yeast cells, so tracking both whole cells and intracellular processes with the bioprobe should be feasible. The most important feature is that the resultant CdSe/ZnS-labeled CMC has good dispersivity. Additionally, the CdSe/ZnS labeled CMC could be used to label intracellular organelles of live cells *via* the functional carboxyl and amino groups of CMC that can conjugate with biomolecules, which are extensively studied nowadays.

In summary, a simple and facile method for preparing CdSe/ZnS-labeled polysaccharides as bioprobes with good dispersivity, uniformity and excellent fluorescence properties has been developed. After incubated with yeast cells, CdSe/ZnS-labeled

CMC was found in the cytoplasm of yeast cells indicating that the bioprobes would be useful for tracking the cells and intracellular processes. Since chitosan used to be a DNA delivery[13] system, the bioprobe of CdSe/ZnS-labeled CMC can be used to study the processes of drug release, and nucleic acid transfection, *etc*. We trust that CdSe/ZnS-labeled polysaccharides will play an important role in molecular biology, biotechnology and biomedicine.

This work was supported by the National Science Fund for Distinguished Young Scholars (No. 20025311), National Key Program for Nanoscience and Nanotechnology (No. 2003BA310A22), and National Natural Science Foundation of China (Grant Nos. 30170268; 30370404; 20305011; 30370017).

Notes and references

‡ The molecular weight of chitosan is 87 000, and the degree of its deacetylation is 85%. The molecular weight of CMC is 10 000, and the degree of its deacetylation is also 85%, the carboxymethyl is in the 3,6-hydroxyl positions, and the degree of carboxymethyl replacement is 0.6.

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