Preparation of CdSe QDs-carbohydrate Conjugation and its Application for HepG2 Cells Labeling

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In present study, CdSe quantum dots (QDs) were prepared with a novel but simple, effective and exercisable method. Nine different types of carbohydrate molecules were used to modify CdSe QDs. D-mannose (Man)-coated quantum dots were prepared for labeling human hepatoma (HepG2) cells, because of the high expression of mannose receptor (MR) on HepG2 cells. The uptake characteristics of CdSe QDs-Man were investigated in HepG2 cells. The absorption rate result of MTT assay in 48 h suggested the extremely low cytotoxicity of CdSe QDs-Man. The presence of quantum dots was confirmed with fluorescence microscopy. These results were encouraging regarding the application of QDs molecules for early detection of HepG2 cells.

Key Words : CdSe quantum dots, Carbohydrates molecules, HepG2 mannose receptor, Receptor-mediated endocytosis

Introduction

One of the fundamental goals in biology is to understand the complex spatio-temporal interplay of biomolecules from the cellular to the integrative level. To study these interactions, researchers commonly use fluorescent labeling for both in vivo cellular imaging and in vitro assay detection. Currently employed diagnostic techniques such as medical imaging, tissue biopsy and bioanalytical assay of body fluids by enzyme linked immunosorbent assay (ELISA) are insufficiently sensitive and specific to detect most types of early-stage cancers. Moreover, these assays are labour intensive, time consuming, expensive and don’t have multiplexing capability. In recent years, QDs have been widely used as versatile inorganic probes, because the detection of QDs is rapid, easy and economical. The unique properties of QDs make them ideal for detecting tumors, including intense and stable fluorescence for a longer time, resistance to photobleaching, large molar extinction coefficients, highly sensitive detection, and that they have narrow, symmetric emission spectra due to their ability to absorb and emit light very efficiently. QDs exhibit strongly size-dependent optical and electrical properties. The ability to join the dots into complex assemblies creates many opportunities for scientific discovery.

The toxicity of QDs is a major problem when they were used in vivo. For example, by ultraviolet irradiation, CdSe QDs will decompose, release toxic Cd to medium, lead to acute toxicity in cultured cells, because the capacity of ultraviolet radiation is fairly close to the covalent bond energy of CdSe QDs.

However, CdSe QDs wrapped with stable polymer shell are relatively stable under ultraviolet conditions. In recent years, a common surface shell coating for CdSe QDs is ZnS, which is used as a shell to prevent them from releasing toxins. After adding a ZnS shell, the mortality rate of cells can be reduced by nine times, and the fluorescence intensity will be higher than that of common CdSe QDs. Furthermore, mercaptaoacetatic acid, polyethylene glycol and other substances could be made as the shell of CdSe QDs. The hydroxy compounds are used to prevent oxidation of CdSe QDs.

In this study, CdSe QDs were coated with nine different types of carbohydrate molecules, which formed carbohydrate shells on their surface. A novel design of multivalent mannoses targeting the MR had been accomplished. In addition, the fluorescence intensity and the toxicity changes of CdSe QDs were carefully observed when they labeled HepG2 cells. CdSe QDs-carbohydrate conjugation provided a new method for surface modification and toxicity study of QDs.

Experimental Section

Materials. Se powder, NaBH₄, CdCl₂·2.5H₂O, D-mannose, D-galactose, L-fucose, D-arabinose, D-glucose, glucuronic acid, D-thannose, D-xylene and sucrose were purchased from Shanghai Reagent Company (China). Thioglycolic acid was the product of Tianjin Institute of Precision Science (China). HepG2 human hepatoblastoma cells were obtained from ATCC (HB-8065, Rockville, MD, USA). All reagents used were of analytical reagent grade.

Preparation of NaHSe. 1 mL of deionized water and 0.1 g of NaBH₄ were added to a round bottom flask, before gently oscillating at the bottom to ensure adequate dissolving. Then 0.079 g of Se powder was added to the flask. The flask was oscillated until the reactant was changed from black to milky. At last, the reaction was carried out at 4 °C for about 2 h with vigorous magnetic stirring.

Preparation of CdSe QDs. 0.0125 mL of thioglycolic acid and 125 mg of CdCl₂ powder were added to 250 mL round bottom flask. After the flask was filled with N₂, 110 mL of N₂-saturated water was added through a syringe.
Then, 0.25 mL of NaHSe was added to 90 mL aqueous solution under a constant temperature of 96 °C for 2 h.

**Preparation of CdSe QDs-Carbohydrate Conjugation.** 5 mL of CdSe QDs were dissolved in phosphate-buffered saline (PBS), pH 7.2. 100 mg each of the nine different types of carbohydrates and 5 mL 1-[3-Dimethylamino propyl]-3-ethyl carbodiimide HCl (EDC.HCl) were added to the buffer. After standing for 4 h at room temperature, the supernatant was removed by centrifugation at 5000 rpm for 10 minutes. After then, the precipitate was redissolved in a small volume of PBS.

**Application of CdSe QDs-carbohydrate Conjugation for HepG2 Cells Labeling.** HepG2 cells were added to DMEM medium supplemented with 100 IU L⁻¹ penicillin, 100 mg L⁻¹ streptomycin, and 10% neonatal calf serum. All cells were inoculated into 96-well culture plates with 100 µL per well. The plate was placed in CO₂ incubator under 37 °C, 5% CO₂ and saturated humidity. When the cells entered logarithmic growth phase, they were digested and counted. The concentration was adjusted into 1 × 10⁶ L⁻¹, and then these cells were inoculated into 24-well culture plates with 100 µL per well. Respectively, nine different types of CdSe QDs-carbohydrates conjugation were added to 18 wells of the plate and the concentration of them was adjusted to 10⁻³ mol mL⁻¹. DMEM medium was added to the remaining wells as negative control. After 24 h of culture, the cells were photographed by using a fluorescence microscope.

**The Toxicity of CdSe QDs-Man in HepG2.** The cells were kept in logarithmic growth phase by trypsin digestion and reincubation every 2-3 days. Trypan Blue Stain was used to distinguish viable cells from nonviable ones. The cell concentration was diluted to 2 × 10⁶ mL⁻¹ with DMEM medium. A 50 µL drop of cell suspension was added into each well of 6 multiwell plate. Then, CdSe QDs-Man were added to each well and the final concentration was adjusted to 10⁻⁵ µg mL⁻¹. CdSe QDs and DMEM medium were used as positive and negative controls, respectively. Cells were incubated in 5% CO₂ at 37 °C, and determined apoptosis after culture for 4, 8, 24 and 48 h, respectively. A 15 µL drop of MTT (5 mg mL⁻¹) was added into each well. After being cultured for 4 h, a suspension of cells was mixed with 100 µL DMSO and counted by the measurement of A570 nm using an ELISA reader DG5032.

**Construction of Mouse Tumor Model.** HepG2 cells obtained from the mouse after the formation of ascites tumor were prepared for in vivo study in ascites. Cell suspension of HepG2 with the density of 10⁷ mL⁻¹ was prepared. At last, 0.2 mL of the cell suspension was subcutaneously injected to the right armpit of the mouse.

**Application of CdSe QDs-Man for Mouse Tumor Model.** 0.2 mL of 1 × 10⁻³ mol L⁻¹ CdSe QDs-Man were subcutaneously injected to the location of the tumor. After 24 h, the mouse was killed and tumor cells were removed.

**Results and Discussion**

**Design of CdSe QDs.** Nanoparticles invariably were prepared as described above and characterized by transmission electron micrograph (TEM), zeta potentiometer and fluorescence spectra (FS). CdSe semiconductor nanoparticles were synthesized in aqueous solution by using thioglycolic acid as the stabilizer. The nanocrystals exhibited a strong, stable and high quality luminescence. TEM images were taken using JEM-2100 operated at 200 kV and the magnification of 150000. The average particle size estimated from the TEM was about 10 nm. As shown in Figure 1, CdSe QDs exhibited a spherical shape, good dispersion, uniform particle size distribution.

**Determination of CdSe QDs-Carbohydrate Conjugation.** As shown in Figure 2 and Figure 3, this fluorescence change was believed to reflect CdSe QDs connected with nine different types of carbohydrates molecules. The excitation wavelength was 435 nm. The fluorescence emission peak of QDs was obtained at 510 nm. After being coated with carbohydrates molecules, fluorescence emission intensity became significantly lower by insulin of carbohydrates. In addition, the maximum of the fluorescence emission spectrum exhibited a marked blue shift compared with...
the maximum emission wavelength for the free QDs. There was no oxidation protection when CdSe QDs-Carbohydrate conjugation was prepared. The majority of the carboxyl groups which were not connected with carbohydrate would fall off and the reduction of carboxyl groups would cause a significant blue-shift as a result of the change in electronic configuration of the QDs.

**Determination of CdSe QDs-Man Signed HepG2 and its Toxicity.** G-block of Olympus BX51 fluorescence microscope is used to observe the HepG2 cells. Under fluorescence microscope, only CdSe QDs-Man were visible in nine different types of CdSe QDs-carbohydrate conjugation (Figure 4). It was due to that, HepG2 and HeLa cells, myelomonocytic cell line, peripheral blood monocyte and alveolar macrophage expressing mannose receptor were positive for the receptor message. This result suggested that only QDs-Man could get into the cells by the mannose receptor of HepG2. In addition, quantum dots were also modified by Man to reduce cytotoxicity into HepG2 for labeling. In primary cultured HepG2, CdSe QDs-Man did not show cytotoxicity during 48 h by MTT assay (Figure 5).

**Signing of HepG2 Cells Using CdSe QDs-Man in Mouse Tumor Model.** In most cases, injection of quantum dots into the tumor yielded rapid transfer of fluorescence into adjacent lymph nodes, visible through the skin. After using CdSe QDs-Man to tag HepG2 cells in live mice, tumors were removed and cut into 1-2 mm pieces. Pieces of tumors were filtered through a 200-mesh screen. Tissue fragments were digested with trypsin and the supernatant fractions were removed. Cell suspension was progressively diluted in PBS and observed under inverted fluorescence microscope. As shown in Figure 6, CdSe QDs-Man had been applied successfully in tumor cells tracking in vivo. The configuration of distinct endocytic vesicles was clearly visualized under a fluorescence microscope.

**Conclusion**

Fluorescence labelling had become increasingly popular as a viable alternative to radiolabelling for many different areas. The fluorescence of the protein was very weak and easily quenched. In addition, the synthesis procedure was complicated. However, CdSe QDs showed excellent properties. In this study, a novel method was provided, which was mediated by thioglycolic acid for conjugating CdSe nanoparticles synthesized in aqueous solution to carbohydrate molecule. The cytotoxicity effects of CdSe QDs were obvious lower after being wrapped with carbohydrates molecules. The method presented in this paper was simple, but effective and feasible.
It was reported that HepG2 cells strongly expressed a large number of mannose receptors. Our finding suggested endocytosis of the CdSe QDs-Man mediated by mannose receptor. Therefore, CdSe QDs-Man might be a candidate for the new HepG2 cells detecting agent in the future.

In summary, the commodious synthesis and application of the CdSe QDs-Man allowed the study of various carbohydrate-protein interactions. Moreover, the cytotoxicity of quantum dots became extremely lower by carbohydrate molecule modification. As a valuable carrier, CdSe QDs-carbohydrate conjugation would be widely used to exhibit its characteristic.

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References