CdTe Quantum dots Modified by Polyamidoamine Dendrimers for Cell Imaging

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Abstract: Water soluble CdTe quantum dots have shown potential as a platform for development of live cell imaging, but their cytotoxicity limits their biological applications. To decrease their cytotoxicity, an approach to modify CdTe quantum dots (QDs) with polyamidoamine (PAMAM) was employed by forming covalent bonds between them. The resultant nanocomposites as a cell imaging bioprobe for live PK15 and their *in vitro* toxicity were also investigated. The results showed that the prepared nanocomposites were able to enter PK15 cells with good biocompatibility and low cellular toxicity.

Keywords: Quantum dots, Dendrimer, Nanocomposites, Luminescence, Cell imaging

Introduction

Rapid advances in nanotechnology and nanoscience have spurred interests in developing a variety of nanostructure materials. In this context, semiconductor nanoparticles (also known as quantum dots, QDs) are the most promising ones due to their high photochemical stability and size-tunable photoluminescence¹. QDs have potential applications in optoelectronics, biosensing, biolabelling and cell imaging^{2,3}. QDs can be linked to biomaterials such as monoclonal antibodies, peptides, oligonucleotides by self-assembly through noncovalent interactions. However, this linking method has inherent instability drawback under such environmental conditions as low pH, higher temperature *etc.* Thus, an alternative approach to preparing QDs assemblies by covalent bond is desirable. In addition, the cellular toxicity of QDs is also of great concerns. Therefore how to enhance QDs' stability and biocompatibility and decrease their toxicity in bio-system is a great challenge⁴.

Polyamidoamine (PAMAM) dendrimers are synthetic spherical macromolecules with well-defined surface, including a core, branches and a lot of terminal groups⁵. Their excellent biocompatibility and low toxicity lead to many potential biological applications⁶. PAMAM dendrimers are small enough to pass into the cell membrane and can be used to deliver substances such as drugs, genetic materials or chemical markers and therefore make them potentially useful for many biological applications^{7,11}. This inspired us to prepare PAMAM dendrimers modified CdTe quantum dots to counteract the defects of QDs.

In this paper, we succeeded in preparing the PAMAM dendrimer-modified QDs through covalent binding (Scheme 1) and investigated their biocompatibility and cytotoxicity in PK15 cells. Our results showed that the QDs could enter into PK15 cells and scattered in the cytoplasm with lower cytotoxicity and good biocompatibility.

Scheme 1. The synthetic route used to prepare the QDs-dendrimer nanocomposites

Experimental

Water soluble CdTe QDs solution was prepared as literature with some modification¹². An aqueous solution of Cd²⁺ ion and TGA was prepared and pH was adjusted to 11. Then, Under vigorous stirring, trisodium citrate, Na₂TeO₃, were added in sequence, after stirring 5 min, NaBH₄ powder was added under open-air conditions. Typical molar ratio of Cd:Te:TGA was 2:1:5 in our experiments. The resulting mixture solution was heated to 100 °C and refluxed to 5 h for obtaining the red CdTe QDs. The CdTe QDs precipitates were rinsed with ethanol and dried by vacuum oven and then were stored at 4 °C in dark.

Synthesis of CdTe–PAMAM nanocomposites

The PAMAM of generation 3.0 (NH₂ terminated) were obtained from Sigma Aldrich, China Mainland. In a typical synthesis of CdTe-dendrimer nanocomposites, 3 mL aqueous solution of CdTe QDs (5.3×10⁻⁶ M) was mixed with 1 mL dendrimers (7.09×10⁻⁶ M) and 100 μL 0.1 mg·mL⁻¹ EDC. The solution was allowed to react at room temperature for 24 h and dialyzed against the phosphate buffer.

The cell imaging and MTT assay

The cell imaging

The PK15 cells were plated onto 20 mm glass coverslips in a 6-well tissue culture plate and were allowed to grow for 24 h in a DMEM medium supplemented with 10% FBS and 1% penicillin streptomycin at 37 °C. The culture medium was then removed, the cells were washed

three times at 4 °C using wash buffer (PBS with 1 mM CaCl_2 and 1 mM MgCl_2) and fixed 20 min with 4% paraformaldehyde solution. Then washed three times with PBS and incubated for about 30 min with 200 μL CdTe-PAMAM dendrimer nanocomposites. After that the cells were washed three times with cold washing buffer and then immediately subjected to imaging under an inverted Laser scanning confocal microscope (Zeiss LSM 510 META).

MTT assay and cell counting

Colorimetric MTT (3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide) assays were performed to assess the metabolic activity of cells treated with CdTe–PAMAM nanocomposites. After the treatment, the medium was removed and replaced with serum-free media (100 μL /well). A total of 10 μL stock MTT (5 $\text{mg}\cdot\text{mL}^{-1}$) was added to each well, and the cells were then incubated for 1 h at 37 °C. The medium was removed and the cells were lysed with DMSO. The absorbance was measured at 490 nm. Cell number was determined by trypan blue exclusion assay.

Results and Discussion

Characterization of CdTe–PAMAM nanocomposites

We successfully prepared fluorescent CdTe-PAMAM dendrimer nanocomposites through covalent bonds by crosslinking agent EDC (Figure 1), then the nanocomposites was characterized by TEM images, photoluminescence (PL) spectra. As shown in Figure 2, transmission electron microscopy images show the good stability and dispersion. Fluorescence emission intensity map shows a slight decrease compared with the quantum dots, but the emission spectrum still shows typical Gaussian distribution (Figure 3).

Figure 1. TEM images of CdTe QD–PAMAM nanocomposites

Figure 2. The normalized luminescence spectra of CdTe QDs before (solid line) and after conjugation with dendrimer (dash line)

Figure 3. Confocal images of fixed PK15 cells incubated with CdTe-dendrimer nanoprobe

The left row represents the fluorescence image, the central row represents the phase contrast image and the right row is an overlay of the left and central rows. CdTe-PAMAM dendrimer nanocomposites were dispersed in the cytoplasm.

The cell imaging

In our study, to evaluate the biocompatibility and cytotoxicity of CdTe-PAMAM dendrimer nanocomposites, we firstly measured the compatibility of as-prepared nanocomposites to PK15 cells. As shown in Figure 4, PK15 cells incubated with CdTe-PAMAM dendrimer nanocomposites, exhibited a strong red color and could not be washed away, which indicated that the CdTe-PAMAM dendrimer nanocomposites could pass into the cell membrane and scattered in the cytoplasm. Then after incubation of PK15 cells with nanocomposites, cell viability was assessed by the MTT assay. The results indicated cell viability was about 75.28% after 48 h incubation; while after incubation PK15 cells directly with QDs for 12 h, the cell viability was only about 25.23%, which proved that the CdTe-PAMAM nanocomposites had no significant cytotoxicity.

Conclusion

In summary, we have successfully synthesized dendrimer-modified CdTe nanocomposites. The CdTe-PAMAM nanocomposites retained the optical properties of the original QDs and they could pass through the cell membrane into the cell with excellent biocompatibility and low cytotoxicity, which may be useful for tracking cells and intracellular processes.

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