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ORIGINAL PAPER

Cadmium sulfide quantum dots modified with the human transferrin protein siderophiline for targeted imaging of breast cancer cells

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Abstract We describe the fairly easy preparation of thiol stabilized water soluble cadmium sulfide quantum dots and the modification of their surface with the human transferrin protein siderophiline. The particles are shown to enable targeted imaging of human breast adenocarcinoma cell (type MCF7). The fluorescence quantum yield of the modified QDs is ~0.74. The particles have an average diameter of 8.1±0.1 nm as determined in solution by dynamic light scattering. The cancer cells were imaged by fluorescence microscopy of the QDs which display strong green fluorescenece under 350 nm excitation. A cytotoxicity assay showed 66 and 78 % cell viabilities, respectively, after 24 h of incubation with the QDs and modified QDs.

Keywords Quantum dot · Fluorescence imaging · Cancer cell · MCF7 · Quantum yield · Siderophiline · Cadmium sulfide

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Introduction

Quantum dots (QDs) are nanocrystalline semi-conductors with high quantum yield of fluorescence, and this has attracted the attention of analytical chemists and biologists [1, 2]. Broadband excitation, narrow emission bandwidth, resistance to quenching and photochemical stability are the main features that results in the replacement of the traditional fluorophores by QDs [3]. Although QDs are most often made by using hot colloidal synthesis, which leaves their surface coated with hydrophobic moieties and prohibit their dispersion in biological fluids [4], many researches have been conducted on the synthesis of water dispersible QDs for biological applications [5–10]. QDs can be solubilized in water with thiol compounds such as thioglycolic acid and mercaptoundecanoic acid [11–13]. The functionalization of the QDs is also a crucial step for producing QDs with specific targeting properties and permeability via cell membrane [14]. The aim of the such developed ultrasensitive detection systems is to provide insight into the biological systems [5, 15]. However, the reports show that the labeling of cellular targets with QD-based probes are less effective than organic dyes due to some important technical problems [16]. As the first successful labeling of a specific target in the cell with a QD probe, the cellular actin fibers were labeled with biotinylated QDs, but the labeling signal was relatively weak [3], and there was a doubt on the specificity and brightness in real applications [16, 17]. Wei et al. confirmed the internalization of folic acid-conjugated quantum dots [12] using over expression of folate receptors on cancer cell surface [18, 19] through the receptor mediated endocytosis [20, 21].

We describe the synthesis of cadmium sulfide quantum dots and solubilization in water by mercaptoacetic acid (MAA). The human transferrin protein, siderophilin, was covalently bonded to the MAA capped quantum dots to form

QD-TF probes. The human transferrin receptor is a membrane-bound protein and is highly expressed on malignant cells. The fluorescence imaging confirmed that that the QD-TF probes were successfully entered to MCF-7 breast cancer cells via receptor mediated endocytosis. The fluorescence quantum yield of the QDs and QD-TF probes were studied with a relative method. The cytotoxicity of the synthesized probes was studied by 3-[4,5-dimethylthiazol-2yl]-2,5-diphenyltetrazolium bromide method.

Material and methods

Materials

Sodium sulfide nonahydrate (≥99.99 %), cadmium chloride, mercaptoacetic acid (MAA), sodium borohydride, sodium hydroxide and partially saturated siderophilin (transferrin human) were purchased from Sigma-Aldrich (Sigma-Aldrich Co., Saint Louis, Missouri, USA, www.sigmaaldrich.com). All of other materials and reagents were of analytical grade. Ultrapure water was used throughout the work.

Apparatus

The absorption spectra of the synthesized quantum dots were recorded using Cary 500 UV–vis-NIR spectrophotometer (Varian Co., Australia). The fluorescence spectra of QDs probes were recorded on a Cary eclipse fluorescence spectrophotometer (Varian Co. Australia). Fourier-transform infrared (FT-IR) spectrum was obtained by OPUS software and FT-IR Tensor 27 spectrophotometer (Bruker, Germany, www.bruker.com). The size distribution of the synthesized QDs was measured by Zetasizer NanoZS90 (Malvern Instruments Ltd, Malvern, Worcestershire, UK, www.malvern.com). Herolab Higen 21 centrifuge (Herolab Gmbh, Wiesloch, Germany, www.herolab.de) equipped with AF 72.2 fixed angle rotor (40° angle, maximum radius of 113–126 mm, maximum volume of 1.5–2.2 mL, K-factor of 322–366 and net weight of 6.5 kg) at maximum 21,000 revolutions per minute (54,000 g relative centrifugal force) was used. The detection system consisted of fluorescence microscope Axioplan 2 imaging system (Carl Zeiss, Göttingen, Germany). The enzyme-linked immune sorbent assay (ELISA) was performed by the ELx808™ Absorbance Microplate Reader (BioTek Instruments, Inc., Winooski, VT, USA, www.biotek.com).

Synthesis of cadmium sulfide quantum dots

Cadmium sulfide QDs were prepared by means of a well-known sol–gel method [22–24]. 100 μL mercaptoacetic acid (MAA, $HSCHCH_2COOH$) was added to 20 mL of 50 mmol L^{-1} $CdCl_2$, while the final concentration of MAA was 55 mmol L^{-1}, and the pH was about 2. The pH was raised to 4.5 and then 7 by dropwise addition of concentrated and 1 mol L^{-1} NaOH, respectively. 100 μL of 25 mmol L^{-1} sodium sulfide was added to this solution with rapid stirring, and the final pH was approximately 8.2. The mixture was stirred for 4 h and then centrifuged at 10,000 g for 30 min.

Conjugation with saturated siderophilin (human transferrin)

Two milligrams of transferrin and 0.0635 mg of $NaBH_4$ were dissolved in 1 mL water at room temperature for 1 h. After 30-min incubation at 70 °C, the transferrin was denatured. Afterward, 30 μL mixture containing 2 mg mL^{-1} transferrin was added to 300 μL of 0.002 μmol L^{-1} QDs under stirring at room temperature. After stirring for 2 h, the solution was centrifuged at 10,000 g.

Cell culture and imaging

Human breast cancer MCF-7 cells, were obtained from the Pasteur institute of Iran (Pasteur institute of Iran, Tehran, Iran, www.pasteur.ac.ir). MCF-7 cells (Human breast adenocarcinoma cell) were grown in RPMI 1640 medium supplemented with 10 % (v/v) heat inactive fetal bovine serum (Gibco), 2 mM of glutamine, and 1 % penicillin streptomycin (Invitrogen) solution in incubator at 37 °C with 5 % CO_2 to allow 70–80 % confluence in 24 h. The cells were washed with pre-warmed phosphate buffered saline (PBS) and incubated in a 60 mm culture plate with pre-warmed fresh media for 30 min. The culture plate was incubated with the conjugated QD for 3 h at 37 °C. The cells were washed twice with cold PBS and the fluorescent images were acquired with fluorescence microscope.

Cytotoxicity assay procedure

Several indirect assays are usually used for the assessment of cell viability. These methods are simple and rapid for the examination of large numbers of tests in 96-well plates. These methods are generally based on the measurement of an enzyme activity which displays the general metabolic status of a cell, and are hence suitable for analyzing cell viability or cytotoxicity. One of the most well-known methods is the 3-[4,5-dimethylthiazol-2yl]-2,5-diphenyltetrazolium bromide (MTT) assay based on the formation and colorimetric quantification of an enzyme activity [25–27]. The MTT assay is a measurable colorimetric method based on the rupturing of the yellow water-soluble tetrazolium salt, MTT, to form water insoluble, dark-blue formazan crystals. MTT splitting occurs only in living cells by the mitochondrial succinate dehydrogenase enzyme. The formazan crystals are dissolved by use of an

appropriate organic solvent, usually isopropanol or DMSO, and the absorbance of the out coming solution is measured using a spectrophotometer. The optical density is directly equivalent to the concentration of the formazan, which is in turn proportional to the number of viable cells. In brief, MCF-7 cells (5×10^3 cells/well) were seeded in 96-well plate and incubated for 24 h at 37 °C in a 5 % CO2 incubator (70–80 % confluent). Cells were treated in triplicate with increasing concentrations of quantum dots and incubated for 24 h. Then 20 μL of MTT solution (0.5 mg mL^{-1}, Sigma) was added to each well and the cells were incubated for 4 h in 37 °C. Viable cells reacted with MTT to form purple formazan crystal. After 4 h, the medium containing MTT solution was exchanged with 200 μL of DMSO to dissolve formazan crystal. Cells were incubated and protected from light for 2 h at room temperature, and shaken for 2 min before reading by ELISA reader at 545 nm. The absorbance of each well converted to percentage of cell growth inhibition.

Results and discussion

Size of nanoparticles

Dynamic Light Scattering (DLS) at 633 nm and a scattering angle of 90° was used for measurement of nanoparticle size. The absorption spectrum of the synthesized QDs are shown in electronic supporting material (Fig. S1). The synthesized QDs do not absorb at 633 nm and Zetasizer nano can be used for characterization of the colloid. In this technique, the diffusion of particles causing by Brownian motion is measured and converts to size and a size distribution by the Stokes-Einstein relationship. The ratio of the diameter of the freshly synthesized particles to their diameters after the different time periods from the synthesis time was used as the degree of aggregation of the QDs. Deviation of this ratio from one, indicates the degree of aggregation of QD nanoparticles. The QD's size distribution of the synthesized colloid has been shown in electronic supporting material (Fig. S2). The diameter of the particles in the colloidal solution was 8.1±0.1 nm with polydispersity index of 0.43. Also the colloidal potential of the particles was −42 mV, the zeta potentials below −30 mV and above +30 mV prevents the aggregation of colloidal particles.

Quantum yield calculations

The fluorescence quantum yield (The ratio of absorbed photons to the fluorescent emitted ones) is important for the characterization of fluorescent probes. Due to sophisticated instrumentation for absolute measurements of the quantum yield, the relative measurement method was applied. In this method the relative quantum yield is determined by comparison to a reference fluorophore with a well-known quantum yield. Rhodamine 6G with 525 nm excitation wavelength and 0.95 quantum yield was used as reference material. The fluorescence quantum yield was calculated from the slope of the fluorescence intensity curve versus the absorbance at the same wavelength by this equation:

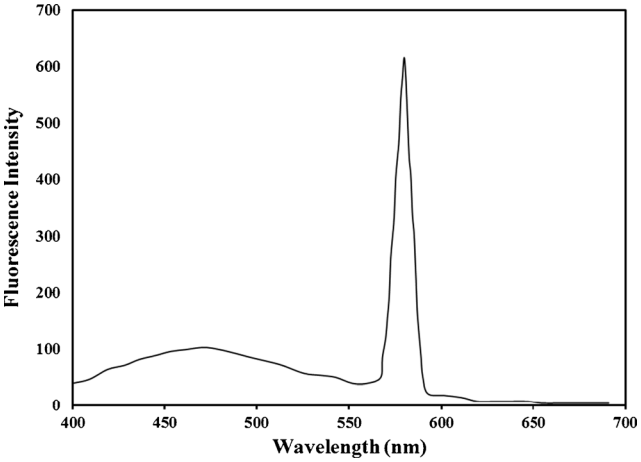

Fig. 1 Photoluminescence spectrum of MAA-capped CdS QD nanoparticles excited at λ_{exc}=350 nm

$$Q_{QD} = Q_R \frac{m_{QD}}{m_R} \frac{n^2}{n_R^2}$$

Where Q_{QD} and Q_R are the QD (or QD-Tf) and Rhodamine 6G (reference) quantum yields respectively. m_{QD} and m_R are the slopes of the lines and are obtained from the plot of the integrated fluorescence intensity vs. absorbance of quantum dots (or QD-Tf) and Rhodamine 6G (reference) respectively. n and n_R are the refractive indices of the solvents used for the QDs and Rhodamine respectively.

Our calculations results in 4272, 3011 and 3374 as the slopes of Rhodamine 6G, QDs and QD-Tf respectively. The

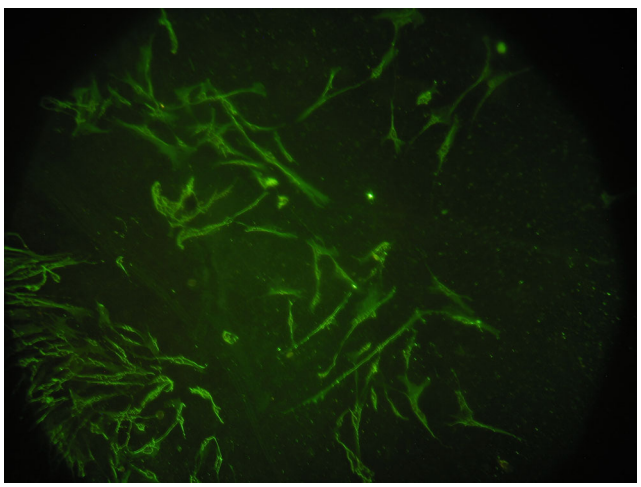

Fig. 2 Fluorescent microscope image of MCF-7 cells that were treated with 50 μL modified QDs. λ_{exc}=350 nm and magnification is 40×

Table 1 Some recently reported flourescent nanoparticles for use in imaging cancer cells

Nanoparticle	Targeting molecule	Cancer	Flourescence intensity at nominal wavelength (A. U.)	Ref.
Gold nanoclusters	Transferrin	Breast cancer	–[a]	[28]
CdSe-ZnS QDs	Prostate antibody	Prostate cancer	~1	[29]
CdS QDs	Folic acid	Liver cancer	~100	[12]
CdS QDs	Transferrin	Breast cancer	600	This work

[a] There is no scale for fluorescence intensity in Fig. 1d of reference [28]

QDs and QD-Tf quantum yields were calculated 0.67 and 0.74 respectively.

FT-IR spectrophotometric study of QDs capped with mercaptoacetic acid (MAA)

FT-IR spectra of MAA-QDs and their pure components (MAA and QDs) were compared to examine the possible interaction mechanisms. The presence of well-defined and strong stretching vibrations of O-H and C=O in MAA-QDs spectrum indicate that the quantum dots have been capped by MAA successfully. The FT-IR absorption spectrum of MAA capped QDs are shown in electronic supporting material (Fig. S3).

Fluorescence imaging of MCF-7 cells

Transferrin receptor is a glycoprotein on the human cell surface for cell proliferation. This receptor is over expressed in malignant than in normal cells due to the higher iron demand for faster cell growth. Based on this fact, transferrin can be potentially utilized as a cell marker for tumor detection. Figure 1 shows the fluorescence spectrum of MAA capped QD nanoparticles excited at λ_{exc}=350 nm. QD-Tf probes were added into the fixed (MCF-7) cells cultured with RPMI (FBS free). The QD-TF and RPMI were mixed adequately and the cells were incubated in a culture box (37 °C, 5 % CO2) for 1 h. The culture solution was then removed, and the cells were washed with PBS three times to remove the excess QDs-TF. Finally, 300 μL PBS was added to the fixed cells for microscope imaging. Figure 2 shows fluorescent microscope image of MCF-7 cells that were labeled by QDs-Tf fluorescent probes (figure S4 in supplementary materials shows the fluorescent microscope image of MCF-7 cells that were treated with unmodified QDs (QDs without siderophiline). Table 1 shows some reported applications of nanoparticles for use in imaging cancer cells [28, 29, 12]. As it is clear, we combine the cancer cell targening power of transferrin with teremendous sensitivity of fluorescent quantum dots for cancer cell imaging. Recently a complete review with good coverage of the nanomaterials used for bioimaging have been published by Wolfbeis [30].

Cytotoxicity of CdS QDs for MCF7 cells

The cytotoxicity of the synthesized cadmium sulfide quantum dots for the MCF7 cell line was performed by MTT viability assay. 1, 2, 4 and 6 micro-liters of the synthesized QD were tested according to discussed procedure. The procedure also repeated for QD-Tf solutions. The absorbances of each well were converted to percentage of cell viability by this formula:

$$The\,percent\,cell\,viability = \frac{Absorbance\,of\,QD\,or\,QD-Tf}{Absorbance\,of\,control\,sample} \times 100$$

figure 3 shows the cytotoxicity of QDs and QD-Tfs with different concentrations for MCF7 cells. The MCF7 cells were treated in triplicates with increasing concentrations of quantum dots and incubated for 24 h in defined medium. The cell viability was calculated as a percentage from the viability of the control (untreated) cells. The viability of the control cells was considered 100 %.

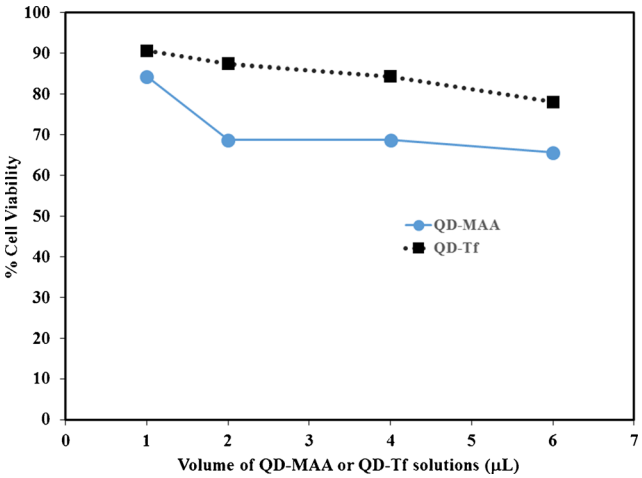

Fig. 3 Cytotoxicity of QDs and modified QDs. MCF7 cells were treated in triplicates with increasing concentrations of quantum dots and incubated for 24 h

Conclusions

Water soluble cadmium sulfide quantum dots were synthesized and conjugated with human transferrin protein. The presence of transferrin molecules facilitate the cellular uptake of modified QDs. This can be due to over expression of transferrin receptors on the fast growing cancer cells. The modified QDs were evaluated for cytotoxicity and successfully applied for targeted imaging of human breast adenocarcinoma cell (type MCF7.

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