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Biofunctional quantum dots as fluorescence probe for cell-specific targeting☆

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ABSTRACT

We describe here the synthesis, characterization, bioconjugation, and application of water-soluble thioglycolic acid TGA-capped CdTe/CdS quantum dots (TGA-QDs) for targeted cellular imaging. Anti-human epidermal growth factor receptor 2 (HER2) antibodies were conjugated to TGA-QDs to target HER2-overexpressing cancer cells. TGA-QDs and TGA-QDs/anti-HER2 bioconjugates were characterized by fluorescence and UV–Vis spectroscopy, X-ray diffraction (XRD), hydrodynamic sizing, electron microscopy, and gel electrophoresis. TGA-QDs and TGA-QDs/anti-HER2 were incubated with cells to examine cytotoxicity, targeting efficiency, and cellular localization. The cytotoxicity of particles was measured using an MTT assay and the no observable adverse effect concentration (NOAEC), 50% inhibitory concentration (IC_{50}), and total lethal concentration (TLC) were calculated. To evaluate localization and targeting efficiency of TGA-QDs with or without antibodies, fluorescence microscopy and flow cytometry were performed. Our results indicate that antibody-conjugated TGA-QDs are well-suited for targeted cellular imaging studies.

1. Introduction

Cancer is one of the leading causes of death worldwide. Early detection of tumor cells can prevent approximately 3.0% to 35% of cancer deaths; thus, it is essential that imaging probes be developed for the early diagnosis of cancer [1,2]. Functionalized fluorescent nanoparticles, such as quantum dots (QDs), are promising probes for biomedicine and cancer research [3,4]. As fluorescent probes, QDs are fundamentally different from organic dyes [5]. Although successful cell labeling has been achieved with organic dyes, they are plagued by low quantum yields and photobleaching. QDs overcome many these problems due to their excellent physical and fluorescent properties [6], including broad absorption spectra, narrow emission spectra [7,8], high quantum yields, resistance to photobleaching, and high photochemical stability [9,10]. Therefore, biocompatible QDs are ideal for cell-labeling studies [11].

Because of the large surface area of QDs, they are readily modified with surface-conjugated biomolecules and proteins [12–14]. QDs are commonly used to image tumor cells after surface labeling with peptides, antibodies, or receptor ligands, such as folate [15]. Guan et al. characterized transferrin-conjugated CdTe/CdSe QDs by different methods and evaluated their cellular targeting capabilities [16]. In another study, RNase-A-associated CdTe QD clusters were coupled to monoclonal antibodies against the human epidermal growth factor receptor 2 (HER2). These theranostic QDs were then used to image and treat gastric cancer *in situ* in a mouse model [17]. Yu et al. reported using GSH-TGA-QDs-ND-1 probes that specifically react with the LEA antigen to target colorectal cancer cells [18]. Geszke-Moritz et al. evaluated the accumulation of folate-conjugated, thioglycerol-capped, Mn-doped ZnS QDs and their subsequent cytotoxicity in T47D breast cancer cells [19]. Recently, Liu et al. synthesized water-soluble indium phosphide/ZnS QDs (QInP) that were functionalized with carboxyl groups and polyethylene glycol (PEG). QinP were then loaded into cells with cell-penetrating peptides (CPP) [20].

Biomedical applications require water-soluble QDs [21]. However, QDs are typically produced in organic solvents, making them

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unsuitable for direct use in biological studies [22]. For cell imaging, the QDs must be transferred into aqueous solutions using ligand exchange reactions, and then conjugated to relevant biomolecules before being incubated with cells [23,24]. Pretreatments, such as ligand exchange, increase the size of QDs, which can make them impracticable for *in vitro* applications [25]. By modification with hydrophilic surface moieties that interact with the aqueous phase, QDs can be rendered water soluble without any additional modification [26].

Antibodies are commonly used targeting moieties because of their diversity and high specificity [27]. For cell imaging, QDs have been modified with cell-targeting antibodies against various antigens, such as the epidermal growth factor receptor (EGFR) [26], Rh-interferon [28], and HER2 [29]. Lung cancer cells often overexpress the erbB (HER) family of oncogenes [30,31]. The c-erbB2 gene encoding the HER2 protein is expressed in 20–30% of non-small cell lung cancers (NSCLCs) and, especially, adenocarcinoma [31,32].

The objective of the study was to design and synthesize fluorescent probes for cell-targeting studies. Water-soluble, thioglycolic acid (TGA)-capped QDs (TGA-QDs) were labeled with anti-HER2 antibodies to specifically image HER2-positive A549 lung cancer cells. Due to the carboxyl groups present on TGA-capped QDs, a simple reaction using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and *N*-hydroxysuccinimide (NHS) conjugated antibodies to QDs. Expression of the HER2 receptor in A549 cells and NIH-3T3 control cells was confirmed by PCR and flow cytometry. Targeting specificity of the TGA-QD/anti-HER2 conjugates was characterized by several methods. Finally, the cellular internalization of QDs was visualized by fluorescence microscopy.

2. Materials and methods

2.1. Materials

All chemicals used here were of the highest purity available. Cadmium chloride ($CdCl_2$, 99%) was ordered from Fluka. Thioglycolic acid (TGA, $HSCH_2CO_2H$, 98%) was purchased from Merck. Tellurium precursor (NaHTe) was prepared from the reaction between sodium borohydride ($NaBH_4$, Riedel 95%) and tellurium powder (Te, Fluka 99.9%). Thiourea (CH_4N_2S, Aldrich 99.5%) was the sulfur source for CdS shell formation. 2-Propanol (C_3H_8O, Riedel 99.5%) was used to purify the nanoparticles. All precursor solutions were prepared using ultra-pure water as a solvent. Rabbit monoclonal antibody against human HER2 protein was obtained from Diagnostic Biosystems. 2-(*N*-morpholino) ethanesulfonic acid (MES), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), *N*-hydroxysuccinimide (NHS), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazoliumbromide (MTT), 4,6-diamino-2-phenylindol (DAPI) and Dulbecco's Modified Eagle Medium (DMEM) were ordered from Sigma Aldrich. Sodium dodecyl sulfate (SDS) and RNAtidy G were purchased from Applichem. Phosphate-buffered saline (PBS) was prepared with 137 mM sodium chloride, 2.7 mM potassium chloride, 10.1 mM disodium hydrogen phosphate and 1.8 mM potassium dihydrogen phosphate, pH 7.4; all chemicals were provided from Sigma Aldrich. The chemicals for protein expression analysis, chloroform, ethanol (75%) and the ingredients of Tris-acetate-EDTA buffer (TAE) that consists of 40 mM 2-Amino-2-(hydroxymethyl)-1,3-propanediol (tris-base), 20 mM glacial acetic acid and 1.0 mM ethylenediaminetetraacetic acid (EDTA), pH 8.0 were purchased from Sigma Aldrich. $Oligo(dT)_{12-18}$ Primer, agarose and Roti-Safe GelStain were ordered from Carl Roth GmbH (Karlsruhe, Germany). The dNTP Set (100 mM solutions) and GeneRuler 100 bp DNA Ladder were obtained from Fermentas. M-MLV reverse transcriptase and its M-MLV RT 5× buffer as well as GoTaq polymerase and its 5× Green GoTaq reaction buffer were provided from Promega (Mannheim, Germany). PCR primers were synthesized by life technologies. Chemicals for native PAGE experiment, ammoniumpersulfate (APS), tetramethylenediamine (TEMED), tris–hydrochloric acid, glycine and bromophenol blue were obtained from Sigma Aldrich. Forty percent acrylamide/bisacrylamide mixing ratio, 37.5:1 was purchased from Carl Roth GmbH. Glycerol was ordered from Fluka.

2.2. Synthesis of water soluble TGA-QDs

In a typical synthesis of CdTe QDs a modified one pot method from the literature was used [33]. Both Te^{2-} and Cd^{2+} precursors were prepared separately. Sodium hydrogen telluride (NaHTe) was prepared by reduction of Te powder with sodium borohydride ($NaBH_4$). Te powder (0.0918 g) and $NaBH_4$ (0.06 g) were put into 25 mL reaction flask and it was purged with N_2 for 30 min. Then 10 mL of deaerated distilled water was added to the reaction flask and the system was heated to 60 °C for 2 h under N_2 atmosphere to obtain a solution with a purple color.

3.12 mmols of $CdCl_2$ and 420 μL of thioglycolic acid were dissolved in 110 mL of ultra-pure water in a two-necked flask with a septum. pH of the solution was adjusted to 11.0–11.5 by drop wise addition of NaOH solution (1.0 M). Then the flask was attached to the condenser under N_2 for an hour at 80 °C to purge oxygen in the medium. The reflux time was one hour. Further reflux before the addition of Te^{2-} precursor may cause decomposition of thioglycolic acid to give S^{2-} [34]. Then 2.5 mL freshly synthesized NaHTe (Te^{2-} precursor) was added to the solution and the reaction temperature was increased to 110 °C.

Formation and growth steps are proceeding upon reflux. As soon as the temperature reaches to 110 °C, sampling was started to observe the growth of the particles. After 10 min, the solution emitted green light under UV-irradiation.

To increase the photostability of the nanoparticles, CdTe NCs were coated with CdS shell. CdS is preferred as a shell material for CdTe cores because band gap of CdS (2.5 eV) is wider than that of CdTe (1.5 eV) and also lattice parameter mismatch between CdTe and CdS is about 3.6% [35].

Proper amount of thiourea dissolved in ultra-pure water was added to green emitting CdTe QDs. The ratio of Te:S related to the amount of applied Te was optimized to 1:10. After addition of thiourea solution, reflux was continued. The prolonged reflux results in red-shifting in both UV–Vis and fluorescence spectra upon coating are indication of core/shell structure formation rather than $CdTe_xS_{1-x}$ alloyed structure. CdTeS alloy structure has larger band gap energy than CdTe and the alloy formation will cause blue-shifting rather than red-shifting. By a changing reflux time, proper sized QDs can be prepared.

2.3. Characterization of TGA-QDs

The synthesized QDs were characterized both optically and structurally. UV–Vis Spectrometer and fluorescence spectrophotometer were used for characterization of optical properties of the nanocrystals. Fluorescence and absorbance spectra of QDs were measured with Varian Cary Eclipse fluorescence spectrophotometer and Varian Cary 50 UV–Vis absorption spectrophotometer. Quantum yield of emission from the QDs is defined as ratio of number of emitted photons to absorbed photons by the QDs. Experimentally the quantum yield can be calculated by absorption and fluorescence emission spectra by comparing fluorescence of both unknown substance and a dye of known quantum yield [36]. Quantum yield of emission from the QD's were measured by using Rhodamine 6G in water as a standard (the reference quantum