PREPARATION AND ANTIMICROBIAL ACTIVITY OF NUCLEOBASE-CONJUGATED CdTe/ZnSe CORE/SHELL QUANTUM DOTS

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Abstract

Inorganic nanomaterials have large specific surface area and high bioactivity which made them promising alternatives to the traditional organic antimicrobial agents that are extremely irritant and toxic. In the present report, CdTe/ZnSe core/shell quantum dots (QDs) were conjugated with different nucleobases and their antibacterial activity against *Escherichia coli* was investigated. The CdTe/ZnSe core/shell QDs were synthesized in a green way or environment-friendly way using water as a solvent instead of organic solvents. They were successfully conjugated with different nucleobases and were characterized using fluorescence and absorbance spectrophotometry and dynamic light scattering techniques. The antimicrobial activity of all the samples along with control (bare QDs) was checked on *E. coli*, a commonly used bacterial model species in microbiology research. Almost all nucleobases showed good antibacterial activity comparing to control probably forming excess ROS which can disturb different metabolisms in bacterial cell. Guanine showed the best result among all other nucleobases.

Keywords: Quantum dots, nucleobases, antibacterial activity

1. INTRODUCTION

Over the past decade, nanoparticles with unique chemical and physical properties have shown an increasing importance in biological, biomedical, and pharmaceutical applications. Inorganic nanomaterials have large specific surface area and high bioactivity which made them good candidates to replace traditional organic antimicrobial agents that are extremely irritant and toxic. In recent years, a number of nanoparticles have been shown to have antimicrobial activities [1, 2] and among them the silver nanoparticles have been well studied and reported to accumulate in the *Escherichia coli* (*E. coli*) membrane to possess effectively antibacterial effects [3]. Silicon dioxide (SiO_2), titanium dioxide (TiO_2), and zinc oxide (ZnO) nanoparticles, also show promising biocidal properties against both Gram-positive and Gram-negative bacteria [4]. All these compounds are found to be photosensitive and can generate reactive oxygen species (ROS) under high-intensity light at a specific wavelength. TiO_2 can be used as a significant antibacterial agent even when sunlight is applied as the excitation source.

In comparison to other nanoparticles, quantum dots (QDs) have better size-dependent optical properties. They are basically nanoscale crystalline clusters synthesized from semiconducting materials [5, 6]. QDs have become more important research topics in recent years [7] due to their unique physical properties including photostability, bright photoluminescence, narrow emission, and broad UV excitation and potential applications in advanced biosensors [8], cell imaging [9] and *in vivo* animal tracking [10]. QDs can show dimensions and numbers of atoms between the atomic-molecular level and bulk material with a band-gap depending on various factors, such as the bond type and strength with the nearest neighbors. Generally narrow and sharp luminescent emission peaks are observed for isolated atoms. It has been reported that a nanoparticle of approximately 100–10000 atoms, shows different narrow optical line spectra. On the basis of this information, QDs can be defined as artificial atoms [11].

It has been reported that under irradiation, QDs generate free radicals, of which the quality and the type are determined by their core materials [12]. The high amount of free radicals is harmful to microbes. The release of free heavy metal ions from QDs could also be toxic to bacteria. Only few of reports on antimicrobial activities of QDs can be found [13, 14]. For example, Kloepfer et al. reported that cadmium selenium (CdSe) QDs can inhibit bacterial growth [15].

To decrease the toxicity of QDs, core/shell structure and environmentally friendly materials are commonly used. Researchers develop different types of core/shell QDs (CdSe/ZnS, CdTe/ZnS, CdTe/ZnSe), core/shell/shell QDs (CdTe/CdS/ZnS, CdSe/CdTe/ ZnSe), and environmentally friendly QDs (CuInSe, Ag_2S, and Si QDs) for various purposes [16-25]. In our previous study, we showed that CdTe/ZnSe core/shell QDs can be applied for *in vitro* imaging of chicken tissue and embryo. In the present experiment, CdTe/ZnSe core/shell QDs were selected as one of the most robust and highly luminescent nanoparticles which are synthesized in a green way or environment-friendly way using water as a solvent instead of organic solvents [26] which are toxic. The CdTe/ZnSe core/shell QDs can be used in different biological fields because the high Cd toxicity of CdTe QDs is expected to be minimized by forming a shell of ZnSe. In the present study, CdTe/ZnSe core/shell QDs were modified with Adenine (A), Guanine (G), Cytosine (C) and Thymine (T) (represented as QDs+A, QDs+G, QDs+C and QDs+T respectively) and subsequently characterized. The antibacterial activities of these core/shell QDs with or without nucleobases modification were checked on E. coli which is commonly used as a model in microbiological research.

2. METHODOLOGICAL BASES AND EXPERIMENTAL PART

2.1. Chemicals

Zinc acetate, cadmium acetate, sodium borohydride, mercaptosuccinic acid and other used chemicals were obtained from Sigma-Aldrich (St. Louis, MO, USA). High purity deionized water (Milli-Q Millipore 18.2 MΩ/cm, Bedford, MA, USA) was used throughout the study.

2.2. Synthesis of CdTe/ZnSe core/shell QDs conjugated with different nucleobases

CdTe/ZnSe QDs were prepared according to our previous reported protocol [26]. Briefly, 10 mL of cadmium acetate (5.32 mg/mL) and 1 mL Mercaptosuccinic acid (60 mg/mL) were added to 76 mL of deionized water and mixed on a magnetic stirrer. Ammonia solution was used to make the pH neutral. Then 1.5 mL of sodium tellurite (221.6 mg/mL) were also added to it and mixed very well. 50 mg of sodium borohydride was added later and the solution was stirred for around 2 h until the bubble formation was stopped. Finally, the volume of the solution was made up to 100 mL with deionized water. Finally the prepared CdTe QDs were stored in dark at 4 °C.

1 mL of zinc acetate (43.9 mg/mL) and 1 mL mercaptosuccinic acid (60 mg/mL) were added to 85 mL of deionized water and mixed properly on a magnetic stirrer. Ammonia solution was used to make the pH neutral. Then 1.5 mL of sodium selenite (5.26 mg/mL) was added to it and mixed for few minutes. 40 mg of sodium borohydride was added later and the solution was stirred for 2 h until the bubble formation was stopped. Finally the volume of the solution was made up to 100 mL with deionized water. The prepared ZnSe QD solution was stored in dark at 4 °C.

Then 1 mL of ZnSe was added to 1 mL of CdTe and heated at a temperature of 50 °C in a closed glass vessel under microwave irradiation (300 W, ramping time 10 min, hold 10 min) (Multiwave3000, Anton-Paar GmbH, Graz, Austria). Then aqueous solutions of nucleobases (A, G, C and T) with a final concentration of 800 μM was added separately to the prepared CdTe/ZnSe core/shell QDs and mixed well in closed glass vessels. In case of control, deionized water was added in place of the aqueous solution of nucleobases. Finally all these samples were heated at a temperature of 95 °C under microwave irradiation using the same conditions as described before. The modified QDs were characterized using fluorescence and absorbance spectrophotometry (Tecan, Grödig, Austria) and Dynamic Light Scattering (DLS) (NANO-ZS, Malvern Instruments Ltd., Worcestershire, U.K.).

2.3. Preparation of bacterial cultures

E. coli (NCTC 13216) was obtained from the Czech Collection of Microorganisms, Faculty of Science, Masaryk University, Brno, Czech Republic. The cultivation media (Luria Bertani) were inoculated with bacterial culture and were cultivated for 24 hours on a shaker at 40 g and 37 °C. The bacterial culture was diluted by cultivation medium to OD_{600} = 0.1 for the following experiments.

2.4. Determination of Antibacterial activities

The antimicrobial effect of the nucleobase-conjugate QDs was determined by measuring the absorbance using an apparatus Multiskan EX (Thermo Fisher Scientific, Germany). In a microtitration plate, *E. coli* culture was mixed with the QDs with or without the nucleobases modification. 250 µL of bacterial cultures was added to 50 µL of the QDs with or without the nucleobases modification and incubated at 37 ºC. The total volume in the microtitration plate wells was always 300 µL. The growth curves were measured for 24 h in 0.5 h intervals.

3. RESULTS AND DISCUSSION

At first, CdTe and ZnSe QDs were prepared separately. After this process, the CdTe/ZnSe core/shell QDs were synthesized under microwave irradiation which was further modified with four different nucleobases. They were observed under UV transilluminator (Fig. 1) and further characterized by fluorescence and absorption spectrophotometry (Fig. 2). The ideal excitation wavelength was 380 nm for the fluorescence characterization. The results indicated that the fluorescent emission spectra were slightly red-shifted after the modification with all nucleobases comparing to control. After the modification with thymine, the fluorescent intensity was significantly increased comparing to control, whereas guanine showed the opposite results.

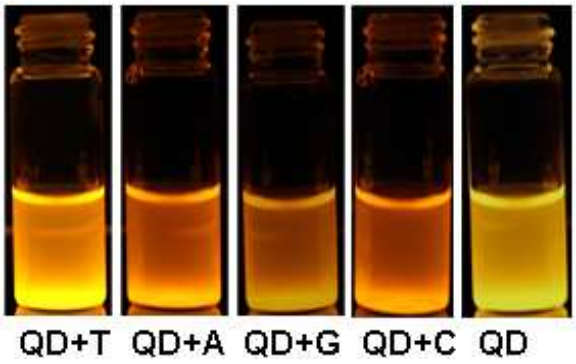

Fig. 1 QDs under UV transilluminator. CdTe/ZnSe QDs conjugated with T, A, G and C are represented as QD+T, QD+A, QD+G and QD+C respectively.

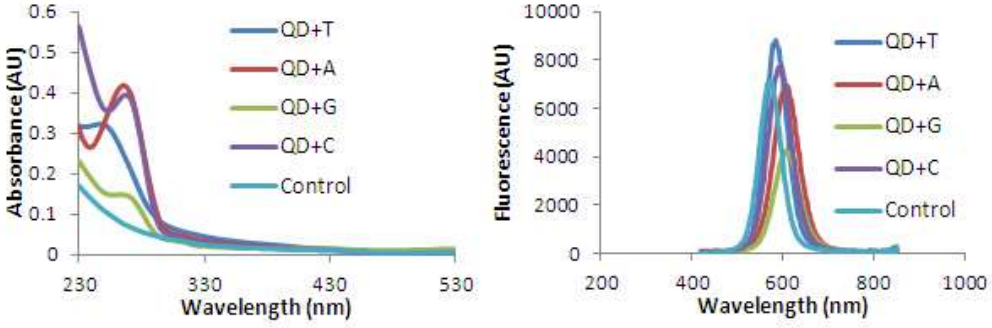

Fig. 2 Absorbance and fluorescence of QDs. CdTe/ZnSe QDs conjugated with T, A, G and C are represented as QD+T, QD+A, QD+G and QD+C respectively. Utilized excitation wavelength was 380 nm within all cases.

The size and zeta potential of the samples were measured using DLS. This technique helped to determine the sizes of QDs with or without the nucleobase modification (Table 1). The results indicated that the sizes of the QDs were significantly increased after the modification with all nucleobases which was in good agreement with the fluorescent spectra. The measurement of the zeta potential (or charge density) was carried out to provide important information for predicting their binding capacity. The result showed that high negatively charges were found in all the samples which indicate that the samples were stable in the solution.

Table 1 DLS characterization of QDs.

Sample	Zeta size	Zeta potential
Control (QD)	10 ± 2	-25 ± 0.11
QD+T	13 ± 3	-30 ± 0.15
QD+A	23 ± 5	-35 ± 0.13
QD+G	31 ± 5	-45 ± 0.16
QD+C	15 ± 3	-32 ± 0.12

The antibacterial activity of QDs (with or without nucleobase-modification) after 24 h was confirmed by the method of the growth curves [27]. All the modified QDs showed significant inhibition of bacterial growth comparing to control bacteria (without addition of QDs) (Fig. 3). From the result, it can be seen that the QDs modified with guanine have the best antibacterial activity against *E. Coli*.

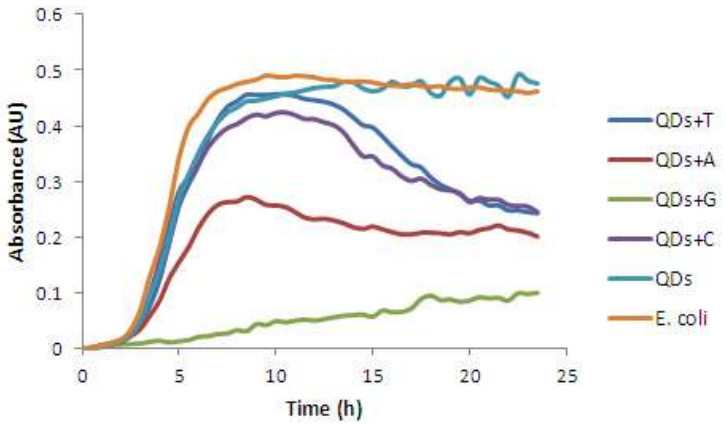

Fig. 3 Antibacterial activity of QDs on *E. coli*. The bacterial growth is represented here by the absorbance. 50 µL of the QDs was added to 250 µL of bacterial cultures and incubated at 37ºC.

4. CONCLUSION

The CdTe/ZnSe core/shell QDs were synthesized in a green way or environment-friendly way using water as a solvent instead of organic solvents. They were successfully conjugated with different nucleobases and characterized using different techniques. The antimicrobial activity of all the samples along with control was checked on *E. coli*, a commonly used bacterial model species in microbiology research. Almost all nucleobases showed good antibacterial activity comparing to control probably forming excess ROS which can disturb different metabolisms in bacterial cell. Guanine showed the best result among all other nucleobases.

ACKNOWLEDGEMENTS

The study was financially supported by CEITEC CZ.1.05/1.1.00/02.0068.

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