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Adherence and interaction of cationic quantum dots on bacterial surfaces

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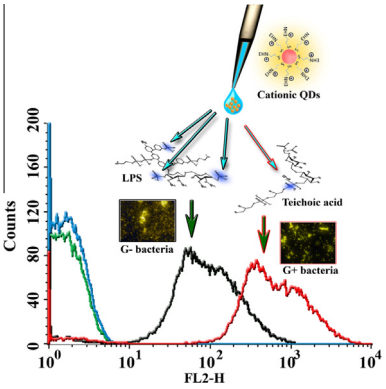
GRAPHICAL ABSTRACT



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ABSTRACT

Understanding molecular mechanisms of interactions between nanoparticles and bacteria is important and essential to develop nanotechnology for medical and environmental applications. Quantum dots (QDs) are specific nanoparticles with unique optical properties and high photochemical stability. In the present study, direct interactions were observed between cationic QDs and bacteria. Distinct fluorescence quenching patterns were developed when cationic QDs interacted with Gram negative and Gram positive bacteria. The aggregation of QDs on bacterial surface as well as fluorescence quenching depends upon the chemical composition and structure of the bacterial cell envelopes. The presence of lipopolysaccharide is unique to Gram-negative bacterial surface and provides negatively charge areas for absorbing cationic QDs. The effects of lipopolysaccharide were proved on fluorescence quenching of cationic QDs. In contrast to Gram-negative bacteria, the presence of teichoic acids is unique to Gram-positive bacterial cell wall and provides negatively charged sites for cationic QDs along the chain of teichoic-acid molecules, which may protect QDs from aggregation and fluorescence quenching. This study may not only provide insight into behaviors of QDs on bacterial cell surfaces but also open a new avenue for designing and applying QDs as biosensors in microbiology, medicine, and environmental science.

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1. Introduction

Nanotechnology has provided a basis for innovation in a wide range of researches and applications. Based on interactions between nanoparticles and cells or biomolecules, novel techniques have been developed and facilitated various biomedical applications including cancer detection [1], drug delivery [2], cell imaging [3], and proteins or bacteria identification [4,5]. Understanding molecular mechanisms of interactions between nanoparticles and cells or biomolecules facilitates developing novel nanotechnology, preventing environmental hazards, and reducing health risks of nanoparticles.

In nature, cells are divided in two broad categories, eukaryotic cells and prokaryotic cells, depending on whether they contain nucleus. Internalization of nanoparticles into eukaryotic cells has been reported through endocytosis. Intracellular transport of nanoparticles has also been observed [6]. In contrast to eukaryotes, prokaryotes such as bacteria do not have an endocytosis system to acquire macromolecules. Nanoparticles may directly interact with and adhere or deposit on bacterial surface [7,8]. The multilayered bacteria cell envelope falls into one of two major groups [9,10]. Gram-negative bacteria are surrounded by two layers of membranes. The outer membrane mainly consists of proteins and lipopolysaccharide (LPS). The presence of LPS is unique to Gram-negative bacteria. Gram-positive bacteria lack an outer membrane but are surrounded by a thick layer of peptidoglycan which is threaded through with teichoic acids, a polymer being unique to Gram-positive bacterial cell wall [9]. When interact with the two types of bacterial surface, environmental substances including nanoparticles may exhibit distinct interaction patterns, which is essential to develop bacteria detectors or sensors [4], microorganisms hybrid devices [7], antibacterial nanoparticles [11], and evaluate environmental risks of nanoparticles [12].

Both types of bacterial surface contain many groups which can deprotonate to have negative charges, and adhere to positively-charged materials [13,14]. Therefore, the electrostatic attraction and repulsion forces can make significant contributions to the interaction between cell surface and positively or negatively charged nanoparticles. Positively charged nanoparticles may possess an interaction mechanism different to negatively charged ones. Cationic nanoparticles such as CTAB-terminated nanospheres and cationic AuNPs can deposit or aggregate on bacterial surface [7,8]. The cationic, hydrophobic, monolayer- protected AuNPs may interact with bacteria through hydrophobic interaction with bacterial surface proteins. The bacterial surface structure is critical to the distinct aggregation patterns of AuNPs on Gram-positive and Gram-negative bacteria [8]. A recent report also showed that the negative or positive charge of nanoparticle would affect cell-membrane interactions [15]. Quantum dots (QDs) are specific nanoparticles with unique optical properties and high photochemical stability, and have already been widely used for cell imaging, molecular labeling and biosensing [16,17]. By taking QDs as representatives, interactions between nanoparticles and cell surface can be investigated with fluorescence techniques. Although scientists have observed interactions between negatively charged QDs and Gram-negative bacteria, there are few reports regarding interactions between positively charged QDs and bacteria [18].

Cysteamine-stabilized CdTe QDs are positively charged in neutral environment and have been reported linking to DNA through amino groups on the QDs surface [19]. In the present study, direct interactions were examined between cysteamine-stabilized CdTe/ZnS core/shell QDs and bacteria. Distinct interaction profiles were observed between Gram-negative bacteria *Escherichia coli* and Gram-positive bacteria *Bacillus subtilis*. Roles of lipopolysaccharides were explored on fluorescence quenching of QDs. The present study focuses on behaviors and fluorescent characteristics of QDs when interact with bacteria, which is essential and important in designing and applying QDs as biosensors in microbiology, medicine, and environmental science. It is also helpful in understanding mechanisms of interactions between similar nanoparticles and bacteria.

2. Materials and methods

2.1. Synthesis of cysteamine-stabilized CdTe and CdTe/ZnS core/shell QDs

Cysteamine-stabilized CdTe QDs were synthesized by a facile one-pot approach using sodium tellurite as the Te source. Typically, 545 mg of cysteamine hydrochloride and 365 mg of $CdCl_2{\cdot}2.5H_2O$ were added to 80 ml of deionized water. The pH was adjusted to 5.7–5.8 with NaOH. Then 17 mg of Na_2TeO_3 and 80 mg $NaBH_4$ were added, followed by heating to 100 °C and refluxing until the photo-luminescence emission peak reached to 560 nm at the excitation wavelength 450 nm. For synthesis of CdTe/ZnS core/shell QDs, 29 mg of $ZnSO4{\cdot}7H_2O$ and 272 mg of cysteamine hydrochloride were added to 40 ml of deionized water. The pH was adjusted to 5.7–5.8 with NaOH and mixed with 40 ml of freshly synthetized CdTe QDs solution, followed by adding 2 mg of $Na_2S{\cdot}9H_2O$. The mixtures were refluxed under 100 °C until the photo-luminescence emission peak reached to 570 nm at the excitation wavelength 450 nm. The QDs solution was stored at 4 °C for later use. Aliquots of the reaction mixture were removed at regular intervals for measuring UV absorption with a UV-2550 spectrophotometer (SHIMADZU) or analyzing photoluminescence with a LS55 fluorescence spectrometer (Perkin Elmer) at room temperature.

2.2. Bacterial strains and culturing

Ten bacterial strains were investigated including *E. coli* DH5α, *B. subtilis* B168, *Listeria monocytogenes* CMCC(B) 54002, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus epidermidis* CMCC(B) 269069, *Proteus vulgaris* CMCC(B) 49027, *Salmonella paratyphi A* CMCC(B) 50093, *Staphyloccocus aureus*, *Bacillus cereus*, *Serratia* sp. All bacteria were cultured in Luria–Bertani (LB) medium with shaking for four to five hours at 37 °C and harvested by centrifugation at 4000*g* for 10 min.

2.3. Cysteamine-stabilized QDs adsorption by bacteria

Bacterial cells were washed and resuspended in 50 mM Tris–HCl, pH 7.0 (Buffer A). Typically, 100 μl of bacteria at specified cell density (OD600) were mixed with 200 μl of freshly prepared cysteamine-stabilized CdTe/ZnS core/shell QDs for 10 min. QDs-labeled bacterial cells were sedimented by centrifugation at 4000*g* for 10 min, followed by and washing with Buffer A, then resuspended in 300 μl of Buffer A for later use.

2.4. Fluorescence spectrometry

The fluorescence spectra of QDs or QDs-labeled bacteria were recorded with a LS55 fluorescence spectrometer (Perkin-Elmer) at an excitation wave length of 450 nm. Bacterial cells labeled by QDs were separated by centrifugation. QDs-labeled bacterial cells and the supernatant were collected and analysed by fluorescence spectrometer, respectively. For monitoring the time-dependent fluorescence quench, 2.7 ml of bacterial cells at density of 0.3 OD600 were mixed with 200 μl of QDs and monitored immediately with a fluorescence spectrometer.