Quantum Dots: Application for the Detection of Salmonella typhimurium

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ABSTRACT

Quantum dots (QDs) or nanocrystals 5 to 50 nm in size have recently emerged as a novel and promising class of fluorophores for cellular imaging. Unlike conventional organic dyes, QDs can be excited by a wide spectrum of wavelengths to give enhanced photostability, and their emission spectra, which differ according to size and material composition, are narrow, symmetrical, and tunable. This novel detection strategy can significantly improve the efficiency of molecular techniques used in the identification of important microbes related to human diseases. The aim of this study was to apply QDs linked antibodies to detect pathogenic Salmonella typhimurium cells. The signal, photostability and efficiency of QDs linked antibodies was compared with the organic fluorophore linked antibody for the detection of Salmonella cells. The use of QDs as fluorophores has shown greater enhancement in photostability and brighter signal than conventional organic dyes in detecting S. typhimurium cells. This approach might also be extended for the simultaneous detection of more than one pathogenic microorganism in the clinical, food or environmental samples by using specific antibodies linked with the QDs of different sizes and colors.

Keywords: antibody, fluorescence, quantum dots, photostability, Salmonella typhimurium

INTRODUCTION

Quantum dots (QDs) are luminescent semiconductor nanocrystals with several significant optical properties and have recently emerged as a promising tool in various fluorescence based biomedical applications in cellular imaging (Bruchez et al. 1998; Chan and Nie 1998; Goldman et al. 2002; Jaiswal et al. 2003; Jurgen and Thomas 2004; Rawn-thorne et al. 2009; Agasti et al. 2010; Byers and Hitchman 2011). The reasons QDs are being preferred over conventional organic fluorophores such as fluorescein isothiocyanate (FITC) or Tetra methylrhodamine isothiocyanate (TRITC) is due to their photostability, size tunability with narrow, symmetric emission spectra and broad continuous excitation which allow excitation of multiple QDs with a single wavelength (Bruchez et al. 1998; Chan and Nie 1998; Goldman et al. 2002; Jaiswal et al. 2003; Jurgen and Thomas 2004). These features make QDs very robust luminescent labels for various biological applications (Kloepfer et al. 2003; Rawsthorne et al. 2009; Agasti et al. 2010; Byers and Hitchman 2011). The use of QDs in applications related to the detection of microorganisms have been conjugated to biomolecules such as streptavidin, avidin, transferrin, immunoglobulin G (IgG), nucleic acids, peptides and wheat germ agglutinin (Chalmers et al. 2007). These QDs and biomolecule conjugates have been shown to bind with enhanced sensitivity to a variety of target microorganisms (Kloepfer et al. 2003; Chalmers et al. 2007; Beckman et al. 2008; Decho et al. 2008).

There have been several reports on the application of QD conjugated antibodies for labeling, detection, and quantification of human oral microbial communities, Mycobacterium bovis, Escherichia coli O157:H7, Salmonella enterica serovar typhimurium, Cryptosporidium parvum, Giardia lamblia and phage-based assays for the detection of slow growing bacteria (Kloepfer et al. 2003; Zhu et al. 2004; Lee et al. 2004; Yang and Li 2005; Yang and Li 2006; Chalmers et al. 2007). These QDs based detection approaches have reported higher detection sensitivity and specificity than that achievable with conventional fluorophores. QDs in a biocompatible form can be prepared by several methods reported in the literature (Bruchez et al. 1998; Chan and Nie 1998; Goldman et al. 2002). Furthermore, QDs are currently available commercially as streptavidin conjugates in a water-soluble form and could be applied for the detection of pathogenic microorganisms if a specific antibody to that particular microorganism is available (Zhu et al. 2004; Lee et al. 2004).

In this study, QDs were applied in labeling and detection of Salmonella typhimurium cells using immunofluorescent antibody based assay. To investigate a QD-based immunofluorescence assay to detect Salmonella typhimurium cells, biotinylated anti-Salmonella polyclonal antibodies (PAb) were conjugated to the streptavidin coated QDs. The photostability of the QDs conjugated antibody was compared with the FITC conjugated anti-Salmonella PAb.

MATERIALS AND METHODS

Microbial strains

All organisms used were purchased from the Microbial Type Culture Collection (MTCC) at the Institute of Microbial Technology (IMTECH), Chandigarh, India. All media and general chemicals were purchased from either Hi-media or Sigma-Aldrich. The incubation temperatures were 37°C for Salmonella typhimurium (MTCC 98) and Pseudomonas fluorescence (MTCC 103). All strains were maintained as pure cultures on Luria-Bertani agar plates and grown with shaking in Luria-Bertani broth at 37°C.

Labeling of bacteria with QDs

Bacterial cells were grown in Luria-Bertani broth under recommended growth conditions until they reached the exponential
growth phase. The biotinylated anti-Salmonella antibodies were polyclonal antibodies raised against the mixture of Salmonella enteriditis, Salmonella typhimurium, and Salmonella heidelberg (Abcam, UK). The Pseudomonas fluorescens strain was used as a negative control in all immunofluorescence based assays.

The strategies used in earlier studies related to the detection of pathogenic protozoans such as Cryptosporidium parvum and Giardia lamblia and used to label Salmonella typhimurium cells (Zhu et al. 2004; Lee et al. 2004). Briefly, biotinylated anti-Salmonella PAb and FITC labeled anti-Salmonella PAb were first reacted with target cells and then conjugation of streptavidin coated QD605 (Invitrogen, USA) was performed to the cells. In detail, 10 μl of target Salmonella cells (~10^5/mL) were spotted on a poly-L-Lysin coated glass slide (Vermicon Identification technology, Munich, Germany). After air dry, 20 μl of blocking buffer (Abcam, UK) was added onto the fixed cells and incubated with 20 μl of QDs solution (dilutions in the range of 5 to 40 nanomoles, nM) for 30 min at 37°C in a humidified hybridization chamber (supplied with fluorescence in situ hybridization (FISH) based kit for the Salmonella detection, Vermicon Identification technology, Munich, Germany). The cells were then incubated with PBS three times each for 5 min, and then incubated with 20 μl of various concentration of QDs solution (dilutions in the range of 5 to 40 nanomoles, nM) for 30 min at 37°C in a humidified chamber. After a final wash with PBS for 5 min three times, the slide mounted with mounting solutions (Abcam and VIT kit) was observed with epifluorescence microscope. Image analysis was performed using ProgRes digital camera system (JENOPTIK, Germany).

In another strategy (Zhu et al. 2004) Streptavidin-QD605 were linked with anti-Salmonella antibodies followed by the reaction with S. typhimurium cells. Diluted preparation of (1X) biotinylated antibodies was first incubated with QDs in the QDs incubation buffer (Invitrogen) for 30 min. After fixing and blocking the target cells, 20 μl of the pre-incubated solution was added onto the slide and incubated at 37°C in a humidified chamber for 30 min. After washing with PBS for 5 min at least three times, the slides were observed under the fluorescent microscope.

Epifluorescence microscopy

An epifluorescence microscope (NIKON eclipse 80i, JAPAN) with a 100W high pressure mercury lamp was used to examine the QD605-streptavidin and FITC anti-Salmonella PAb tagged Salmonella cells. Slides were examined with the filter sets G-2E/C for TRITC and QD605 (excitation wavelength, 540 to 565 nm; dichroic wavelength, 565 nm; emission, 605-655 band pass) and B-2E/C for FITC (excitation wavelength, 465 to 485 nm; dichroic wavelength, 505 nm; emission, 515-555 band pass). Images were captured and analyzed using ProgRes digital camera system (JENOPTIK, Germany).

RESULTS

Detection of Salmonella cells by QDs

The two strategies used for labelling target cells with QDs-antibody bioconjugates are illustrated in Fig. 1. The S. typhimurium cells from the 10-μl of exponentially grown culture (~10^5/mL) were detected with FITC-labeled anti-Salmonella PAb and QD605 conjugated anti-Salmonella PAb. The numbers of S. typhimurium cells were counted by the method described in the literature. The concentration of QD605-streptavidin was increased from 5 to 30 nM and this has resulted in an increased number of Salmonella cells being detected. The detection sensitivities with 10 nM QD605-streptavidin concentration were found to be enough for the detection of a maximum number of Salmonella cells. However, the detection sensitivities achieved at 20 and 30 nM QD605-streptavidin had not affected the number of detected cells. Furthermore, with FITC-labeled anti-Salmonella polyclonal antibodies a higher concentration up to 20-40 nM was required to detect a comparable number of Salmonella cells. Thus, 10 and 30 nM were the optimum concentrations required for the detection of Salmonella cells by QD605-streptavidin and FITC-labeled anti-Salmonella PAb, respectively. Fig. 2 shows epifluorescence images of QD605-labeled Salmonella cells with 5 nM (Fig. 2A) and 10 nM QD605-streptavidin (Fig. 2B). As indicated in Fig. 2, the number of detected Salmonella cells increased by increasing the concentration of QD605-streptavidin from 5 to 10 nM. However, a further increase in the concentration of QD605-streptavidin up to 30 nM had no significant effect on the number of detected cells.

Photostability of QD605-conjugated anti-Salmonella antibodies

The photostability and increased brightness are the main advantages of QDs over traditional organic fluorophores. In several studies in the past, it has been observed that the brightness of QDs could differ for QDs that are conjugated and used in staining experiments (Wu et al. 2003). Both streptavidin-QD605 and FITC-anti-Salmonella PAb labeled Salmonella were continuously illuminated for 3 min with a Nikon epifluorescence microscope fitted with a 100-W mercury lamp. The images were captured with a ProgRes digital camera system (JENOPTIK, Germany) at every 15-s interval, and the intensity was analyzed with the imaging system supplied by Nikon (Japan). In this study, it was noted that QD605-streptavidin labeled Salmonella cells exhibited brighter signals even at the lower concentration of QD605.
The FITC-PAb labeled Salmonella cells have shown good signals at the higher concentration. However, the FITC-labeled Salmonella cells signal bleached significantly after 2 min of illumination. In contrast, it was observed that there was insignificant reduction in the signal for QD605-labeled Salmonella cells. That is, the QD605-labeled Salmonella cells remained photostable even after 3 min of continuous illumination.

DISCUSSION

Salmonella typhimurium has been recognized worldwide as the major cause of most cases of human salmonellosis transmitted through food, water and other environmental sources (Carlson et al. 1999; Sharma and Carlson 2000; Baggesen et al. 2000; Baudart et al. 2000). Rapid and sensitive detection of pathogenic Salmonella strains in various environments is important for preventing the outbreaks of food/waterborne diseases associated with its infection. The detection of Salmonella is usually achieved through specific labels that selectively stain its cells according to its biochemical, structural or nucleic acid properties using antibody-based, oligonucleotide based fluorescent in situ hybridization (FISH) and PCR-based methods (Carlson et al. 1999; Sharma and Carlson 2000; Baudart et al. 2000). Due to their high sensitivity and specificity, fluorescently labeled antibody based probes are most commonly used among all these methods. However, different environmental samples can contain inert objects or living or dead cells that have strong fluorescence and light scatter characteristics similar to, for example, fluorescently labeled pathogenic Salmonella cells, interfering with the detection of Salmonella. Therefore, careful selection of fluorescent dyes is the key to the detection and identification of pathogenic Salmonella strains.

Recent studies have revealed quantum dots (QDs) as a novel and promising class of fluorescent reporting systems for cellular imaging. QDs, which are nano scale inert particles (~5-50 nm), provide much higher photostability than conventional organic dyes and can be excited by a wide spectrum of wavelength from UV to red (Bruchez et al. 1998; Chan and Nie 1998; Goldman et al. 2002; Jiasembl et al. 2003; Klopfer et al. 2003; Jurgen and Thomas 2004; Zhu et al. 2004; Lee et al. 2004; Yang and Li 2005; Yang and Li 2006; Chalmers et al. 2007; Rawsthorne et al. 2009; Agasti et al. 2010; Byers and Hitchman 2011). Since the emission spectra of QDs, which differ according to the size and material composition, are narrow, symmetrical and tunable, the use of QDs as a fluorescence reporting system can potentially and significantly minimize the interference from natural auto fluorescent particles, and provide multiplexing detections on different target cells with a clear discrimination from extraneous particles (Zhu et al. 2004). Recent progress on the conjugation techniques of QDs with biotin, avidin, protein transferring and antibodies have greatly increased the potential of QDs in detecting different molecular targets at different sub-cellular locations of various biological specimens like live cells, fixed cells and tissue sections.

The two strategies used in this investigation were applied for the detection of Cryptospordium parvum and Giardia lamblia in a previous study (Zhu et al. 2004). There was no significant effect observed on the labeling of Salmonella by streptavidin coated QD605 by these two strategies. As suggested in the Zhu et al. (2004) study, in strategy 2, excessive amounts of QDs were needed to reduce the numbers of antibodies bound onto one QD before conjugating the antibodies would bind QDs to cells. As a result, the signal brightness should decrease rapidly with the increase in the dilution of QDs concentration. However, in this study both strategies achieved almost the same signal brightness at the 10 nM QDs working concentration. In addition, an increase in the QD working concentration in both strategies did not affect the brightness of QDs labeled Salmonella cells. This particular observation was inconsistent with the earlier study (Yang and Li 2006) where 10 nM working concentration of QD605 and QD525 failed to generate detectable signal for detection of E. coli O157:H7 and S. typhimurium. This might be due to the different working concentrations of biotinylated antibody used in this study. In addition, this observation could also be attributed to the use of polyclonal antibodies as compared to the monoclonal antibodies used in a study conducted by Yang and Li (2006). The polyclonal antibodies used in this study were targeted at multiple O and H antigens displayed by most of the members of the Salmonella family. The polyclonal antibodies used were able to bind Salmonella cells more effectively by QDs tagged PAb even at the lower concentration of 5nM or 10nM QDs. However, further study is needed to compare the efficiency of anti-Salmonella monoclonal and PAb to label Salmonella cells at different QDs working concentrations. In another closely related study, a 30 nM working concentration of QD655 was found to generate stable signals for the detection of Streptococcus gordoni in oral biofilms (Chalmers et al. 2007). In the current study, 5-10 nM working concentration of QD-605 was found to be sufficient to detect bacteria in pure culture. The effect of various antigen agents on the photostability of QDs was also investigated in this study. These mounting solutions included immersion oil, antifade solutions used in the VIT (Vermicon Identification technology, Munich, Germany) kit, 10% BSA in 1X PBS used in reference 10 and 90% glycerol. The antifade agent supplied with FISH based VIT-Salmonella kit gave the best results. However, there was not much reduction in the signal in the case of QDs labeled Salmonella cells. The immersion oil which was found to be the best antifade mounting solution in the Zhu et al. (2004) study was found to be inadequate in the case of FITC labeled Salmonella cells. Other mounting solutions including 1X PBS and pure glycerol were further tested, and all gave poorer results than that of the VIT kit supplied antifade agent.

CONCLUSIONS

This study successfully demonstrated labeling of Salmonella typhimurium cells with streptavidin-QDs conjugated with polyclonal antibodies against Salmonella. QDs were shown to have increased photostability and brightness over conventional organic dyes in the detection of S. typhimurium cells. This approach might also be extended for the simultaneous detection of different members of the Salmonella family in the same sample by using QDs with different sizes and colors. Therefore, QDs labeling is a novel, promising and durable method for the identification and detection of pathogenic or environmentally relevant microorganisms in clinical, food and other environmental samples.

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REFERENCES


