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A quantum-dot-based fluoroassay for detection of food-borne pathogens

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ABSTRACT

Evaluation of the distribution capability of food-borne pathogens existing in food products by taking the advantage of quantum dots (QDs) for their photoluminescence properties was carried out. Bacteria namely *Escherichia coli* (*E. coli*) labelled with CdSe-QDs were examined both on an Agar nutrient and ground fish substrates in order to observe their growth rate in different environments in the Lab. A sample with an appropriate concentration ratio 10^7 CFU/mL of bacteria/CdSe-QDs was empirically selected from the samples which were grown on the Agar containing plates. The selected sample was also tested on a ground fish substrate as a real food sample. The bacterial growth was observed under the irradiation of UV light and the growth patterns were investigated for 3 successive days. The growth patterns indicated that *E. coli* can stay alive and can be distributed on food products so that the growth can be easily monitored. This approach makes bacterial growth on food products detectable so that it can be used as a bacteria-QD assay for an easy detection of food borne pathogens grown on a food sample.

1. Introduction

Food-borne disease outbreak is a major international problem. Recently, reported food disease, in particular the outbreak of *Escherichia coli*, drew special attention to the importance of performing research on food-borne pathogens and their persistence. Since food-borne disease outbreaks frequently implicate commercially produced food products, they should have great importance from the health and economic points of view. Even though there are strict regulations considered for the case of food production and related packaging industries, the frequency of food-borne disease is growing. For example, in Europe, campylobacter infection was reported as the highest food-borne related disease in humans in 2009.

Usually, pathogens might be available in low numbers in a sample making them unsuitable to identify. Traditional pathogen detection methods like colony count estimation can be time consuming with completion ranging from 24 h for *E. coli* to 7 days for *Listeria monocytogenes* and this causes notable difficulties for semi-perishable foods in order to control their quality. By employing these methods microorganisms numbers can be underrated because microorganisms come into viable but non-culturable states (VBNS) under various environmental stresses.

Upon occurrence of environmental changes and resuscitation from VBNS state e.g. a rise in temperature, the cells can recover the capability of infection and therefore this enables them to result in a health risk.

Amid the methods cited for the detection of food borne pathogens are microbial culture methods which maintain the 'gold standard' for detection [1]. Delicate immunological or molecular-based assays are established due to the demand of rapid recognition of pathogen [2–9]. Illness, however, might be resulted from the presence in food of between 10 and 100 colony forming units (CFU) of the four major food-borne pathogens (*Campylobacter jejuni*, *E. coli* O157:H7, *L. monocytogenes* and *Salmonella enterica* serovar typhimurium). As a result, these low infective doses are taking these assays to their limit so that the need for more sensitive assays seems to be necessary.

Nanobiotechnology, basically, is the junction of nanotechnology and biology. Nanotechnology deals with the characterization, fabrication and manipulation of structures, devices or materials with sizes between 1 and 100 nm [10]. Compared with the bulk form, the nano form of the material provides larger surface to volume ratios and superior electronic and optical properties [11–13]. Progress in the handling and controlling of these nanomaterials allows binding of various biomolecules such as proteins, bacteria, and nucleic acids [14–20]. Using nanomaterials for biosensing offers plenty of advantages, one of which, due to their large surface area, is allowing a greater number of biomolecules to be immobilised and this, therefore, raises the number of reaction sites available for interaction with a target

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species [19]. This characteristic in combination with excellent electronic and optical properties facilitates the use of nanomaterials in 'label-free' detection and in development of biosensors with enhanced sensitivities and improved response times [21]. Food safety standards are becoming more rigorous; hence, the potential of highly sensitive nanomaterial based diagnostic systems to detect a single bacterial cell become an important consideration [22].

QDs deal with the quantum confinement of electrons and hole carriers at dimensions smaller than the Bohr radius. QDs or nanometer-scale semiconductor crystallites mainly consist of elements from groups II and VI such as CdSe, CdTe, and CdS or III and V like InP [30]. QDs, nanoparticles, in which electrons and holes are three dimensionally limited within the exciton Bohr radius of the object, giving to this nanocrystallites unique optical properties e.g. size-tunable photoluminescence color [31]. In addition, bright emission, distinguished photostability, availability in various color, large-surface area, and with NIR photoluminescence make QDs attractive for biological imaging, spotting of pathogenic bacteria, and diagnostics [8,9,31].

Quantum dots are semiconducting fluorescent nanoparticles which are applicable for the detection and reduction of food borne pathogens due to their unique size-dependent fluorescence properties and photostability. In many cases QDs are used instead of traditional fluorescent dyes [1,23]. Further, their application which attracts most interest in terms of food safety assays is their use as labels in immunoassays. So far, QDs have been utilized as fluorescent labels in numerous assays in order to detect food borne pathogens, for instance *L. monocytogenes* [24], *C. jejuni* [25], *E. coli* [26], *S. typhimurium* [27], *S. aureus* [28], and *Shigella flexneri* [29]. QDs in comparison to traditional dyes display brighter fluorescence intensity for longer, and signal amplification has led to the lower detection thresholds. Further, the use of magnetic bead-quantum dot assay for the capture and detection of bacteria can result in quenching of the fluorescent signal [32].

Considering the cost and size of the QDs, there is a disinclination to totally change from traditional dyes to QDs. Although QDs are in the nanometer range, they are still an order of magnitude greater in size than most dyes, possibly resulting in interference with bio recognition in multiplex assays [1]. Recently, materials such as metal, semiconductor, polymer and ceramic nanoparticles have received much attraction in the imaging and PDT (photodynamic therapy) of cancer. Moreover, semiconductor quantum dots (QDs) received much interest as probes for bioimaging [31]. Due to asymmetric and crystal-filed splitting, and mixing of carrier exchange perturbations with angular momentum of the charge carriers, the band-edge states are quantum confined or size-dependent, and are 8-fold degenerate in CdSe QDs. For instance in the case of CdSe QDs the photoluminescence color shifts from near visible to NIR region with an increase in the size of QDs. In CdSe QDs, the highest occupied states are contributed by the 4p orbitals of selenium and the lowest unoccupied states are contributed by the 5 s orbitals of cadmium [31]. Nanoparticles have been widely utilized as signal reporters for detection of biomolecules in DNA assay, immunoassay and cell bioimaging. QDs can also be used as fluorophores in fluorescence in situ hybridization (FISH) [30]. Another major feature of QDs is their distinguishable ability to code substrates with various colors during multiplexing assays [30].

It is essential to understand the potential hazards of nanoparticles on human health and on other living organisms while the use of nanoparticles in different scientific researches and medical applications continues. Although the application of nanoparticles as antimicrobial agents indicates distinguishable results, the safety issues of nanoparticles and their effect on human health is still ambiguous [32]. As long as an explicit understanding of the processes by which nanoparticles eliminate bacteria and their potential health effects is acquired, their widespread use in food and food processing endures unfeasible. Knowing that trace concentrations (≈10–100 cells) of pathogens in food can generate a serious threat to human health. Therefore, it is essential to ensure the safety of food that we consume by recognition of food borne pathogens at all stages of the food production. If the problems related to the prevalent use of nanomaterials such as robust functionalisation techniques and simple cost-effective fabrication can be solved, the potential of these nanobiotechnologies is vast. Nanobiotechnologies together with advances in other technologies such as microfluidics and miniaturised devices can make easy to carry, highly sensitive, convenient diagnostic implements for food-borne detection a reality. Consequently, the "farm-to-fork" follow up of food borne pathogens would become standard for food safety measures. There is a large body of research which their major aim is foodborne pathogen detection. For instance, L. Wang et al. [33] demonstrated an EDC – protein A QD-labeling technique and fluorescence measurement to detect *E. coli* O157:H7 and salmonella in ground beef. Xiuheng Xue et al. [34] were demonstrated a method of detecting live/dead *Staphylococcus aureus* cells which was developed based on CdSe QD-immunoglobulin G and propidium iodide fluorescent labeling. Xiuheng Xue et al. [35] were demonstrated a fluorescence measurement method for rapid detection of two bacteria, *E. coli* and *Staphylococcus aureus* (*S. aureus*), by using water soluble quantum dots (CdSe) as a florescence marker acted as detection apparatus.

In this study, a nanobiotechnology assay was demonstrated for rapid detection of food borne pathogen, *E. coli*, using CdSe water soluble quantum dots as marker. The distribution of bacteria labelled with QDs in different concentration of bacteria/nanoparticle dispersion solutions was studied on Agar nutrient media. In addition, we planned to investigate the distribution of *E. coli* in a food sample of ground fish in order to have an experimental evaluation of a food borne pathogen on a real sample. The selection of appropriate concentration of bacteria conjugated with nanoparticles was performed in order to evaluate the growth rate of *E. coli* on the ground fish using a bacteria-QD assay as an easy detection method during 3 consecutive days. This method provides a path to detect distribution of bacterial cells on a food sample.

2. Experimental

2.1. Materials and Characterization Method

2.1.1. Material

Na_2SO_3 (≥ 97%), selenium powder (≥ 99%), cadmium acetate dehydrate (≥ 99%), oleic Acid (≥ 99%), ethanol (95.1–96.9 V%) and N-hexane (≥ 90%) were prepared from Merck, Darmstadt, Germany. NaOH (≥ 98%) was purchased from Arman Sina Company, Iran. For all of the experiments ultrapure water was used. All materials and solvents were used as received. Luria Bertani broth (99%) and Luria Agar (LA) (99.5%) were prepared as nutrients from Merck, Darmstadt, Germany. Microbial strains of *E. coli* (ATCC 11303) were received from the lab of Persian Type Culture Collection, Iran. Ultrapure water was used throughout the bacterial preparation procedure. 1-ethyl-3-(3-dimethyllaminopropyl)-carbodiimide (EDC) (≥ 99%) used as activator was prepared from Merck, Hohenbrunn, Germany. Reagents including crystal violet ($C_{25}N_3H_{30}CL$, 96.0%), iodophor (99.9%), safranin ($C_{18}H_{15}N_4CL$, 98%), and ethanol (C_2H_5OH, 95.1–96.9 V%) were prepared from Merck, Darmstadt, Germany. Other inorganic reagents utilized for preparation of phosphate buffered saline (PBS) including sodium chloride (NaCl, 99.5%), potassium chloride (KCl, 99%), disodium hydrogen phosphate dehydrate ($Na_2HPO_4{\cdot}2H_{2O}$, 99.0–100.5%), and potassium dihydrogen phosphate (KH_2PO_4, 99%) were prepared from Merck, Darmstadt, Germany.

2.1.2. Characterization Method

A Varian Carry Eclipse UV–Visible Spectrometer was used to investigate photoluminescence property of the samples under the irradiation of UV light. Photoluminescence emission spectra were obtained by using a 6305 Jenway Spectrofluorometer. The samples were centrifuged by using a Hettich EBA 20 centrifuge. The growth of bacteria was performed in an Incubator 124 OD. Colony Counter devices were