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RAPID MONITORING OF BACTERIA BY FLUORESCENT MICROSCOPY USING WATER SOLUBLE QUANTUM DOTS

Tapas K. Mandal*, Nargish Parvin, Mitali Saha

Department of Chemistry, National Institute of Technology, Agartala, Tripura- 799055 India

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For Correspondence:
Tapas K. Mandal
Department of Chemistry, National Institute of Technology,Agartala-799055,India

E-mail:

tps.mndl@gmail.com

ABSTRACT

Our work is to demonstrate a fluorescence measurement method for rapid detection of bacteria and their counting by using water-soluble carbon quantum dots (CQDs) as a fluorescence marker, and fluorescence microscope acted as detection apparatus, while Escherichia coli (E. coli) was as detection target bacteria. Highly luminescent water-soluble CQDs were prepared by carbonizing waste plant materials (different types of grasses, shrubs, herbs etc) in a furnace under in-sufficient air flow, and were then covalently coupled with target bacteria. E-coli in a LB media with < 0.1 um of water soluble CQDs were cultured for 1-2hrs. The multi colored bacterial cell images were obtained by using fluorescence microscopy. Our results showed that CQDs prepared in water phase were highly luminescent, stable, and successfully conjugated with E. coli. The fluorescence method could detect the E. coli after 1-2 hours cultured with water soluble CQDs. Also we could count the total number of bacteria per unit within a short time.

INTRODUCTION:

Rapid and sensitive detection of bacteria is extremely important in biotechnology, medical diagnosis, and food safety. Because of slow detection speed and complicated procedures, plate count, as a conventional method, could not ideally meet the requirements of fast and efficient microbe detection any more. Recently, several new methods like ELISA, PCR, diffraction based cell detection, and flowcytometry detection have been reported.[1,2] However, most of current available methods for detecting trace amounts of bacteria need either amplification or enrichment of the target bacteria in the sample, and moreover the apparatus are expensive.[3,4] Therefore, simple and sensitive methods, which do not need target amplification or enrichment, are required.

In this study, a fluorescence measurement method was demonstrated for rapid detection of bacterial count using water-soluble CQDs as marker, fluorescence microscopy as detection apparatus and Escherichia coli (DH5 alpha) as detection target bacteria.

MATERIALS AND METHODS

Synthesis of water-soluble CQDs:

C-dots were prepared by carbonizing waste material of plants in a furnace under in-sufficient air flow. The black mass so obtained was grounded in a mortar and then was treated with concentrated nitric acid. On overnight standing the acid was diluted with water and the black mass was allowed to settle and the supernatant liquid was decanted off. This process was repeated several times to free the mass from nitrate (checked by Greiss's reagent).The black mass was then dispersed in distilled water by sonication and filtered through a Whatmann-1 filter paper followed by cellulose acetate filters with 0.1 µm pore size. The filtrate was evaporated under vacuum to yield the desired water soluble carbon dots as black powder.[5] Figure-2 shows AFM and TEM images of WSCQDs.

Bacterial cell cultured with water soluble CQDs:

Microbial strains of E. coli were collected from the lab of Dr. R. Grantham (Indian Institute of Technology Kanpur, India). They were cultured in 20mL of Luria Bertani (LB) broth and 200µl of quantum dot solution was mixed to use as an aliquot (0.5mg/ml conc.). The mixture was made under sterile condition and in a sterile conical flask. After incubation 1to 2hrs at 37 ◦C, the bacterial cells were centrifuged for

30sec at 18000 rpm. The bacteria were washed thrice with the sterilized PBS (pH 7.4) and then resuspended in100 µl of PBS (pH 7.4) under gentle vortex mixing for fluorescence microscopy (LEICA DC200). We hypothesized that CQDs could be put into use as an additional measure of cell viability.

Fluorescence microscopy:

Images of bacteria were captured by using a Leica inverted microscope (Leica DC200, Leica microscopy system ltd, CH-9435, Heerbrugg) with an attached RS Photometrics Sensys camera, KAF1401E G1. The intensity of fluorescence was quantified by using the 488, 561 and 633nm band pass (BP) emission filter functions of the Leica microsystem imaging solution software (Leica Q fluoro version V1.0a, Leica microsystem imaging solution ltd, Germany).

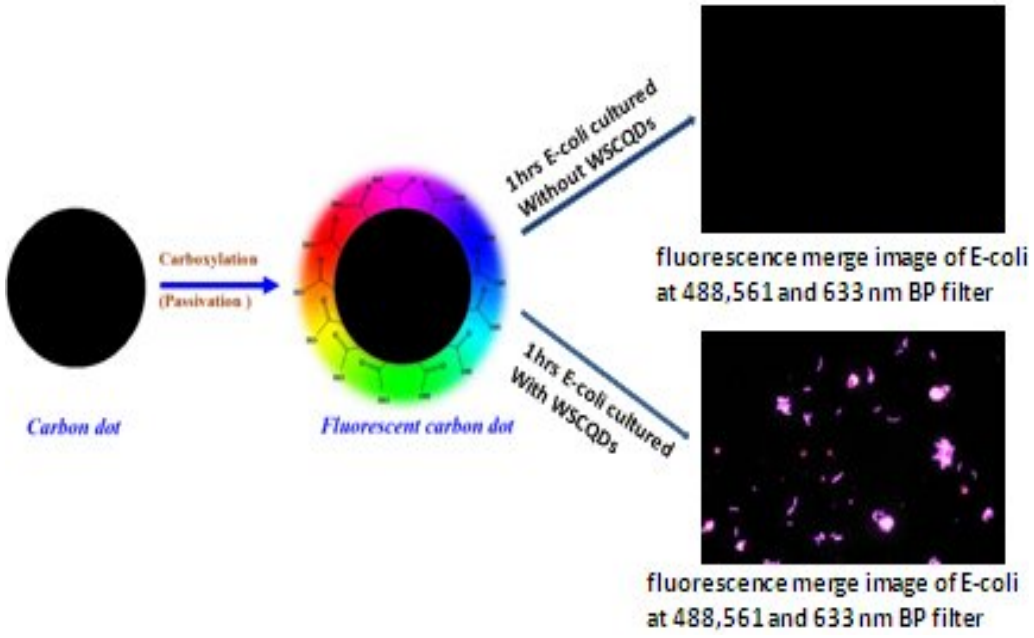

FIGURE. 1. Scemetic diagram of fluorescence carbon dots and non fluorescing and fluorescing E-coli which cultured 1hrs ,without and with WSCQDs respectivly.

RESULTS AND DISCUSSION:

In order to identify whether the water-soluble CQDs bind to the bacterial cells, in this experiment, E. coli was chosen as detection target bacteria because they possess different cell walls and the component which was well understood. The water soluble CQDs 200uL (0.5mg/mL) and E-coli were coupled together within 1-2 hrs only.

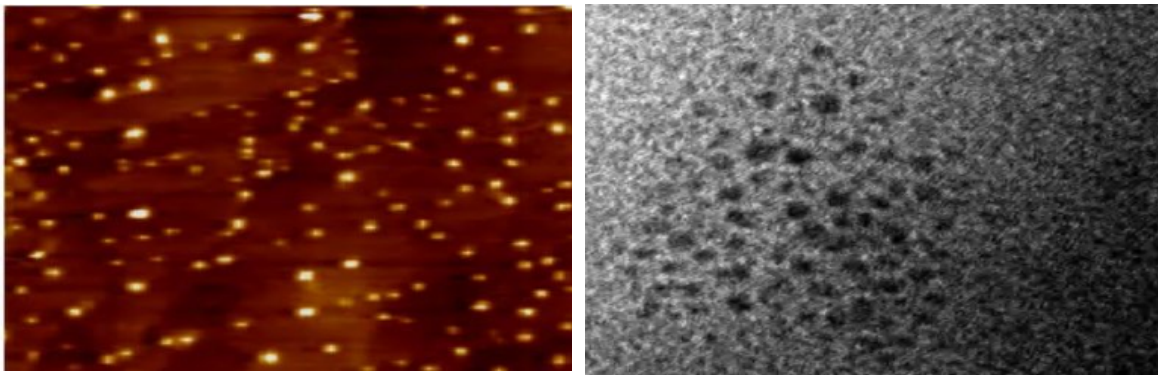

FIGURE 2. AFM topography images of up) C-Dots and HRTEM image C.Dots.

The fluorescence microscopy images demonstrate that the fluorescence signals of the E. coli cells coupled with water-soluble CQDs were clearly seen approximately 1 to 2 hrs after addition of the CQDs solution to the bacterial culture broth. A multi color luminescent E. coli cells were observed under a fluorescent microscope (Figure-3).

In contrast, the fluorescence signals of the E. coli cells without addition of the CQDs were invisible in control experiments. It also showed that CQDs bind on the surface of E. coli cells. Furthermore, these images reveal water-soluble CQDs bind on the E. coli. The possible mechanisms are that the water-soluble CQDs with surface carboxylic acids bind on the surface of Skin cells.[6,7] But another published paper indicated that QDs are able to label to bacteria only if the particles are <5 nm in diameter. They through that the diameter of the particles was about 3–4nm could pass through bacterial cell walls and membrane entry the cell, so the QDs could label to bacteria. But the luminescent bacterial cells were observed approximately 12 h after addition of the QD solution to the bacteria in log phase.[8]

In this study, we used CQDs as a fluorescent labeling to explore whether the water-soluble CQDs bind on the surface of bacterial cells. As shown in the fluorescence signals of the bacterial cells with addition of the water soluble CQDs are clear and bright, but the fluorescence signals of the bacterial cells without addition of the water soluble

CQDs become invisible. It indicated that the water-soluble CQDs bind on the surface of bacterial cells. Since the surface of CQDs were the functional carboxylic Group was free, which can be easily coupled with amine groups the surface of bacteria, such as proteins, peptides and amino acids. In fact, one bacterial cell membrane carries numerous proteins and one protein typically bind on numerous water soluble CQDs conjugate. Briefly, the detection was based on coupling amino group of bacterial cell membranes with water soluble CQD bio-labeling. This fluorescence method could detect the total count of E. coli in a short time that of at least 20–30 times lower than conventional plate counts.

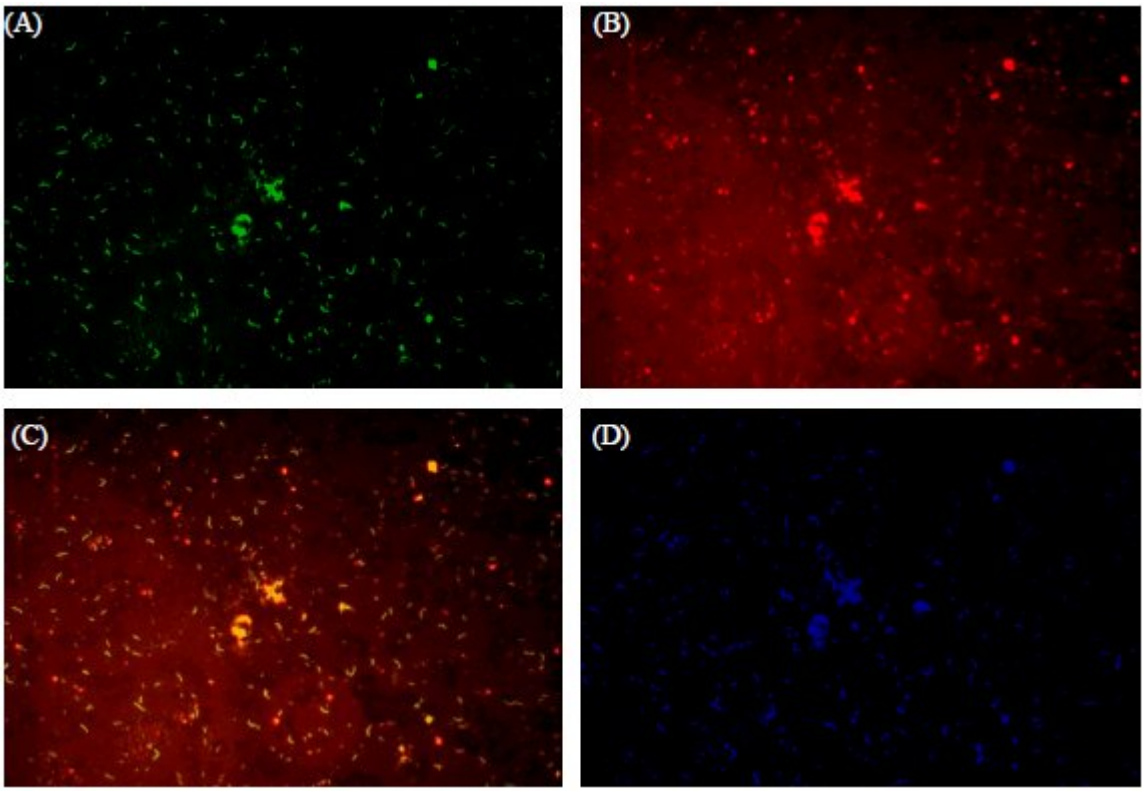

FIGURE. 3. Fluorescence images of E-coli after 2 hours cultured with < 1 um sized water soluble carbon quantum dots (WSCQDs) under fluorescence microscope. Images observed under (A) 488nm,(B) 561nm and (C) merged of A&B and (D) 633 nm band pass filter.

CONCLUSION:

This study has prepared highly luminescent and stable water soluble CQDs. A sensitive and rapid fluorescence detection method of total bacterial count has been demonstrated based on the water-soluble CQDs quantum dots coupled with bacterial. The fluorescence method could detect of E. coli in a short time per unit. Also this method can be used in numerous environments, including the air, water, clean rooms and aseptic isolators of various factories/Companies. This method can also be used to rigorous field tests by independent and government testing facilities. Further, a new fluorescence technique has been established to determine the microbial content of a sample.

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