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Employing carbon dots modified with vancomycin for assaying Gram-positive bacteria like *Staphylococcus aureus*

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ABSTRACT

By employing attractive performance of fluorescent carbon dots, we herein successfully established an assay for analyzing bacteria firstly. Specifically, carbon dots with blue fluorescence were initially synthesized according to a previous report, and modified with vancomycin on their surfaces. Subsequently, the prepared carbon dots were applied to detect *Staphylococcus aureus* accompanied with a linear range of 3.18×10^5–1.59×10^8 cfu/mL as well as a detection limit of 9.40×10^4 cfu/mL. Compared with other regular methods, our method is more rapid and convenient in term of methodology. Meanwhile, the current strategy was applied for detection of other bacteria including *Bacillus subtilis*, *Listeria monocytogenes*, *Salmonella*, *Pseudomonas aeruginosa* and *Escherichia coli*, and the modified carbon dots showed obvious affinity with Gram-positive bacteria owing to the ligand–receptor interactions between vancomycin and the cell walls, suggesting its value for detecting Gram-positive bacteria. Additionally, the practicability of this sensing approach was validated by recovery experiments conducted in orange juice, confirming its potential to broaden avenues for detection of Gram-positive bacteria.

1. Introduction

Staphylococcus aureus (*S. aureus*), belonging to a member of Gram-positive bacteria, is also well known as a virulent pathogen blamed for its highly stabile toxic proteins mainly including different staphylococcal enterotoxins and the toxic shock syndrome toxin (Arbuthnott et al., 1990; Balasubramanian et al., 2007). More importantly, *S. aureus* can cause diverse diseases such as pneumonia, pericarditis, urinary tract infections and even septicemia. Such infections could be lethal with improper medical treatment, so it has been considered to be one of the most serious risks in the public health all around the word. Remarkably, *S. aureus* is temperature (7–48 °C) and pH (4.2–9.3) tolerant and can survive in hypersaline environment (Jia et al., 2014; Pividori and Alegret, 2010; Wang et al., 2011a). Especially, foods like milk, dairy products and meat products are easily contaminated by *S. aureus* once treated in unclean environment. Hence, quantification detection of *S. aureus* exhibits great significance for human safety. Currently, various efforts have been devoted to explore convenient and accurate approaches for identifying bacteria (Chan and Chen, 2012; Chen et al., 2008b; Lin et al., 2013; Xie et al., 2012; Yang et al., 2010), but each of them showed its own drawbacks. On the basis of routine bacterial culture and isolation technology, bacterial enrichment in medium followed by plate counting served as the traditional and most common method (Jia et al., 2014), and was obviously limited by time consuming and low sensitivity. Another common method by virtue of commercial optical scanning devices is also time-consuming (24 h) for incubation and is not suitable for clinical diagnosis, although it can provide satisfactory detections of bacteria (Gao et al., 2006). Besides, the detection technique originated from real-time fluorescent quantitative PCR (FQ-PCR) has been employed for detecting the fluorescence of specific nucleic acids in *S. aureus*. However, the similar disadvantages of requiring highly skilled operators and expensive equipment, being prone to contamination and providing indirect relationship between the results and concentrations of *S. aureus* also limited its applications (Ding et al., 2011; Thet et al., 2013). Consequently, traditional assays towards pathogen are obviously defective, lead to the necessity of developing rapid and sensitive strategies.

Carbon dots (CDs) are fascinating carbon materials owing to their unique properties consisting of stable photoluminescence, tunable excitation and emission wavelength, lack of optical blinking, small size, low toxicity, favorable biocompatibility and satisfactory fluorescent performance (Baker and Baker, 2010; Wang et al., 2011b; Yang et al., 2009a). Hence, this kind of material have drawn considerable attentions in the fields of nanobiotechnology, biological labeling, photocatalysis (Cao et al., 2011; Li et al., 2010), sensing (Zhao et al., 2011; Zhou et al., 2012),

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biomedicine (Wang et al., 2011a) and delivering drugs (Lin et al., 2005; Yang et al., 2009b). In the past few years, two major approaches have been developed for synthesizing CDs. One way was known as the top down, consisting of electrochemical oxidation (Zhao et al., 2008; Zhou et al., 2007), acidic oxidation (Dong et al., 2010), arc discharge (Xu et al., 2004) and laser ablation (Hu et al., 2009). For the other way, hydrothermal (Zhu et al., 2012), microwave (Salinas-Castillo et al., 2013) and ultrasonic (Zhuo et al., 2012) defined as the bottom up, have been basically applied to synthesize CDs. Compared with conventional organic dyes and semiconductor quantum dots (QDs) that are seriously pernicious for human health and the environment, CDs exhibited fascinating properties and potentiality not only in theoretic but also in practical aspects. Especially, numerous CDs have been utilized to design fluorescent probes towards analysis purpose (Algarra et al., 2014; da Silva and Goncalves 2011; Goncalves et al., 2010).

Serving as one of glycopeptide antibiotics, vancomycin (Van) usually functions as a broad-spectrum antibiotic, and was first isolated from a soil sample collected by an American pharmaceutical company of Eli Lilly in the mid-1950s (Williams and Bardsley, 1999). Currently, the vancomycin group antibiotics have been propelled to the front of fighting against the serious pathogen like *S. aureus* and MRSA (methicillin resistant *Staphylococcus aureus*) on the basis of their clinical effect for treating Gram-positive bacterial infection (Xing et al., 2002). By forming hydrogen bonds, vancomycin can bind to the terminal peptide of D-Ala-D-Ala on the cell wall of the Gram-positive bacterium (Dancer et al., 1996; Gu et al., 2003; Liu et al., 2011; Williams and Bardsley, 1999). In fact, substantial contributions have been devoted to test bacteria by utilizing the broad affinity between vancomycin (Gao et al., 2006; Gu et al., 2003; Lin et al., 2005; Qi et al., 2013), lectins (Afonso and Fenselau, 2003; Bundy and Fenselau 1999; Bundy and Fenselau, 2001), immunoglobulin G (Chen et al., 2008a; Ho et al., 2004) and bacteria.

Inspired by the attractive fluorescent performance of CDs and the interactions bacteria binding with vancomycin, we hereby established a facile approach for fast and accurate detection of Gram-positive bacteria originated from CDs modified with vancomycin (Fig. 1). Specifically, the modified CDs aggregated on the surfaces of bacteria owing to the interactions between vancomycin and bacteria, thereby facilitating the decrease of CDs' fluorescence. In contrast with traditional methods, this solution largely shortened the detection time to less than 1 h and allowed the whole process with scarce interference from complex samples. Accordingly, we have provided a promising opportunity to help overcome the difficulties faced by conventional detection methods, which generally requires time consuming and sophisticated operations (Liu et al., 2011). Additionally, the practicability of this sensing approach was validated by recovery experiments of detecting *S. aureus* in orange juice, and the results demonstrated its potential for analyzing Gram-positive bacteria existing in multi-matrix. Meaningfully, the current strategy by means of carbon dots conjugated with vancomycin (CD@Van) for detecting Gram-positive bacteria was reported for the first time.

2. Materials and methods

2.1. Chemicals

N-hydroxysulfosuccinimide (NHS) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) were purchased from Sigma-Aldrich (Milwaukee, USA). Vancomycin hydrochloride was obtained from Shanghai Sangon Biotech Co., Ltd (Shanghai, China). Disodium hydrogen phosphate (Na_2HPO_4), sodium dihydrogen phosphate (NaH_2PO_4), citric acid, urea, sodium chloride (NaCl) and sodium hydroxide (NaOH) were purchased from Dingguo Changsheng Biotech Co., Ltd (Beijing, China). Tryptone, beef extract, peptone, yeast extract powder, ager were obtained from Beijing Aoboxing Biotech Co., Ltd (Beijing, China). *S. aureus*, *Salmonella*, *Pseudomonas aeruginosa* and *Escherichia coli* were acquired as gifts from microbiology laboratory, College of Pharmaceutical Sciences, Southwest University (Chongqing, China). *Bacillus subtilis*, *Listeria monocytogenes* (*L. monocytogenes*) were purchased from China General Microbiological Culture Collection Center. Ultrapure water of 18.25 MΩ cm, produced with an Aquapro AWL-0502-P ultrapure water system (Chongqing, China), was employed for the following experiments.

2.2. Instrumentation

All fluorescence measurements were performed on a Hitachi F-7000 fluorescence spectrophotometer (Tokyo, Japan) with excitation slit set at 5 nm band pass and emission at 5 nm band pass in 1 cm × 1 cm quartz cell. High resolution transmission electron microscope (HR-TEM) images were taken via a TECNAI G2 F20 microscope (FEI, America) at 200 KV. Meanwhile, UV/vis absorption spectra were recorded by a Shimadzu UV-2450 spectrophotometer (Tokyo, Japan). Functional groups analysis was achieved by Fourier Transform Infrared spectrometer (Tokyo, Japan). Mass spectra were obtained by UltrafleXtreme Matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry (MALDI TOF/TOF MS, Bruker Daltonics Inc., USA). A Fangzhong pHS-3C digital pH meter (Chengdu, China) was used to

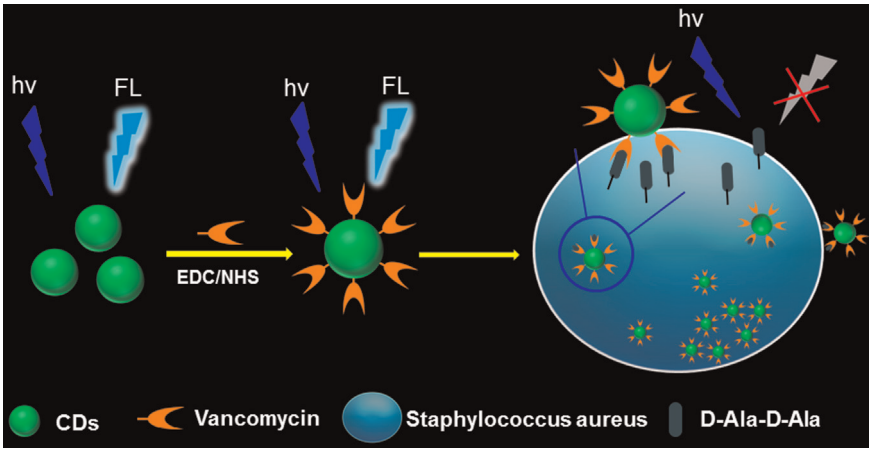

Fig. 1. Schematic illustration of our method for detecting *Staphylococcus aureus*.