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Rapid and sensitive detection of *Staphylococcus aureus* assisted by polydopamine modified magnetic nanoparticles

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ABSTRACT

Pathogens cause significant morbidity and mortality to humans. Thus, development of fast and reliable methods for detection and identification of pathogens is urgently needed to increase protection level of public health and ensure the safety of consumers. Herein, a rapid and sensitive method has been developed for *Staphylococcus aureus* (*S. aureus*) detection based on the dual role of polydopamine modified magnetic nanoparticles (PDA@Fe_3O_4 NPs) combined with polymerase chain reaction (PCR) and capillary electrophoresis (CE). The core-shell type structure PDA@Fe_3O_4 NPs were prepared, which are spherical, about 152 ± 20 nm in diameter and the PDA shell is about 17.5 ± 1.6 nm. PDA@Fe_3O_4 NPs play a dual role including efficient capture of bacteria and extraction of DNA. In the pH range of 3.0–7.0, the capture efficiency of *S. aureus* by PDA@Fe_3O_4 NPs was more than 95% in 5 min. The adsorption capacity of the PDA@Fe_3O_4 NPs for *S. aureus* is 1.2×10^8 cfu mg^{-1}. The efficient capture and concentration of bacteria from large volumes of samples by PDA@Fe_3O_4 NPs avoids the time-consuming culture-enrichment prior to PCR. Interestingly, PDA@Fe_3O_4 NPs were also found to be efficient adsorbents for extraction of genomic DNA from pathogens based on the electrostatic interaction. The process can be finished in 25 min. The PDA@Fe_3O_4 NPs based solid phase extraction combined with PCR and CE allows for detecting the order of 10^2 cfu mL^{-1} *S. aureus* in tap water and orange juice samples. The whole process takes < 5.5 h. The developed method would provide a promising platform for rapid and sensitive detection of pathogens.

1. Introduction

Pathogenic bacteria are widely spread in our daily life and natural environment, and pose a great world-wide threat to food safety and public health. For example, *Staphylococcus aureus* (*S. aureus*) is carried by up to 30–50% of humans in nostrils and on skin surfaces [1]. *S. aureus* can cause numerous diseases, from minor skin and soft tissue infections to life-threatening illnesses, such as abscesses, pneumonia, meningitis, endocarditis, and septicemia [2]. Therefore, development of rapid and reliable methods for detection and identification of pathogens is urgently needed to increase protection level of public health and ensure the safety of consumers.

The traditional method for detection of bacteria is based on culture and colony counting, which has good reliability and high sensitivity [3]. Nevertheless, the method is extremely lengthy because it usually requires several days to obtain a result. In order to address this deficiency, many researchers have paid a great deal efforts to develop various methods for rapid detection of bacteria, such as polymerase chain reaction (PCR)-based methods [4–6], immunology-based methods [7–10]. In immunology-based methods, preparation and purification of antibody is still a dispiriting task. PCR-based methods are popular due to the rapidity, reliability, as well as high specificity. Nevertheless, the limits of detection for bacteria are commonly in the range of 10^3–10^4 cfu mL^{-1}. In order to detect low concentrations of bacteria (< 10^3 cfu mL^{-1}), a lengthy culture-enrichment step is often still required prior to PCR. Thus, it is still a challenge to develop new PCR-based methods for rapid and sensitive detection of bacteria.

Magnetic particles (MPs) have attracted great attention in biological analysis because of their large surface-to-volume ratio, unique magnetic response and easy surface modification [11–15]. MPs modified by some particular groups, such as antibody [16], aptamer [17], antibiotics [18], amino acid [19] and carbohydrates [20] have been implemented for pathogens capture. The MPs-based solid phase extraction (MSPE) methods can be combined with the PCR-based methods to improve the detection sensitivity of bacteria since the MSPE methods can preconcentrate bacteria, thus avoiding the time-consuming culturing step. Recently, we reported preparation of *N*-methylimidazolium functionalized MPs (MIm-MPs) and cationic polyelectrolyte functionalized MPs

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(PDDA-MPs), which were used as adsorbents for pathogens capture and combined with PCR and capillary electrophoresis (CE) to rapidly detect low concentrations of pathogens in the order of less than 10^2 cfu mL^{-1} [21,22]. In these methods, the pathogens captured by these MPs needed to be eluted from these MPs. Then the DNA template was extracted from the eluted pathogens for PCR. We tried to directly extract the DNA template from the captured pathogens by MPs. However, the PCR was not successfully accomplished since DNA was also adsorbed by these MPs and MPs interfered with PCR. In order to further shorten the assay time for bacteria, we expect to prepare a new type of MP, which can capture pathogens and avoid the elution step prior to extraction of DNA template and PCR.

Dopamine is an attractive functional monomer, which can be self-polymerized under alkaline conditions to form a firmly hydrophilic polydopamine (PDA) film on a wide variety of materials [23,24]. Recently, PDA modified magnetic nanoparticles (PDA@Fe_3O_4 NPs) were found to enable capture and release of DNA based on the charge switch of phenolic hydroxyl and amino groups on the surface of PDA@Fe_3O_4 NPs adjusted by pH [25]. In this study, the capability of PDA@Fe_3O_4 NPs as absorbents for rapid capture of *S. aureus* was investigated. Based on the dual role of PDA@Fe_3O_4 NPs for the capture of pathogens and DNA extraction, a method for rapid and sensitive detection of *S. aureus* in tap water and orange juice samples was developed.

2. Experimental

2.1. Materials and chemicals

Ferric chloride hexahydrate ($FeCl_3{\cdot}6H_2O$), ethylenediamine tetraacetic acid disodium (EDTA), ethylene glycol (EG), diethylene glycol (DEG), ethanol and sodium acetate anhydrous (NaAc) were all supplied by Tianjin Damao Chemical Reagent Factory (Tianjin, China). Sodium acrylate (Na acrylate) was obtained from Xiya Chemical Industry Co., Ltd (Linyi, China). Dopamine hydrochloride was supplied by Alfa Aesar (Shanghai, China). Sodium dihydrogen phosphate dihydrate ($NaH_2PO_4{\cdot}2H_2O$), disodium hydrogen phosphate dodecahydrate ($Na_2HPO_4{\cdot}12H_2O$), sodium chloride (NaCl) and tris (hydroxymethyl) amino methane (Tris) were supplied by Guangzhou Chemical Reagent Factory (Guangzhou, China). Sodium dodecyl sulfate (SDS) was supplied by Guangzhou Genebase Bioscience Company (Guangzhou, China). 2-(*N*-morpholino) ethanesulfonic acid (MES) was supplied by Sigma (Saint Louis, MO, USA). Hydroxypropylmethylcellulose (HPMC), the viscosity in 1% aqueous solution at 25 °C was 4000–6500 mPa s, was supplied by Shanghai Sangon Bio-Tech Co., Ltd (Shanghai, China). Water (18.2 MΩ cm) generated by an ELGA water purification system (London, UK) was used throughout the experiments.

Taq DNA polymerase, 10 × PCR buffer, $MgCl_2$, and deoxynucleotide triphosphate (dNTP) mixtures were supplied by Takara Bio-Tech Co., Ltd (Dalian, China). A GeneRuler 100-bp DNA ladder including 10 fragments ranging from 100 to 1000 bp (total concentration 0.5 mg mL^{-1}) was supplied by Thermo Scientific (Waltham, MA, USA). The primers in PCR experiments were synthesized and purified by Shanghai Sangon Bio-Tech Co., Ltd (Shanghai, China).

2.2. Synthesis and characterization of PDA@Fe_3O_4 NPs

The synthesis of PDA@Fe_3O_4 NPs involved two steps. Firstly, carboxyl modified magnetic nanoparticles (Fe_3O_4-COOH NPs) were synthesized via a solvothermal reduction method previously reported [26]. Briefly, $FeCl_3{\cdot}6H_2O$ (1.08 g) was dissolved in a mixture of DEG (30 mL) and EG (10 mL). Then NaAc (3.0 g) and Na acrylate (3.0 g) were added and the mixture was vigorously stirred to form a homogeneous dark yellow solution. Afterwards, the mixture was transferred to a 50 mL Teflon-lined stainless-steel autoclave and heated at 200 °C for 10 h. At last, the black products were washed thrice with ethanol and deionized water, followed by drying at 60 °C with nitrogen stream for further use.

Secondly, PDA@Fe_3O_4 NPs were synthesized. Firstly, dopamine solution was prepared by dissolving dopamine hydrochloride (40 mg) in Tris-HCl buffer (40 mL, 10 mM, pH 8.5). Next, Fe_3O_4-COOH NPs (10 mg) were added to the dopamine solution and heated at 40 °C for 3 h. Finally, the obtained PDA@Fe_3O_4 NPs were washed with deionized water thrice, followed by drying at 40 °C with nitrogen stream.

The TEM images of Fe_3O_4-COOH NPs, PDA@Fe_3O_4 NPs, and the conjugates between PDA@Fe_3O_4 NPs and *S. aureus* were performed on a JEM-1400PLUS transmission electron microscope (JEOL, Tokyo, Japan). The FT-IR spectra were recorded on an iS50 FT-IR spectrometer (Nicolet, Madison, WI, USA). The zeta potentials of Fe_3O_4-COOH NPs and PDA@Fe_3O_4 NPs were measured by a Zetasizer Nano ZS (Malvern, Worcestershire, UK).

2.3. Preparation of bacteria samples

The *S. aureus* (CMCC26003) was cultured in nutrient broth (0.15 g beef extract, 0.25 g bacterial tryptone, 0.25 g NaCl, 50 mL sterilized water, pH 7.0) at 37 °C and 200 rpm for 10 h on a rotary shaker. Bacteria were harvested by centrifugation (5000 rpm, 5 min) and then separated from the broth. The separated bacteria were washed twice and resuspended in phosphate buffer (PBS) (10 mM, pH 7.0). The absorbance of different concentrations of bacteria at 600 nm (A_{600}) were measured on K5600 micro-spectrophotometer (Beijing Kaiao Technology Development Company, Beijing, China). The bacteria concentration expressed as colony forming units (cfu) was acquired through the plate counting method. All the containers were sterilized in an autoclave at 121 °C for 30 min before and after use.

2.4. Magnetic capture of S. aureus with PDA@Fe_3O_4 NPs

First, the bacteria concentration was adjusted to a certain level (A_{600}, 0.4) using PBS (10 mM, pH 7.0). Then 1 mg PDA@Fe_3O_4 NPs were added to 1 mL bacterial suspension (A_{600}, 0.4). The mixture was incubated at room temperature by shaking at 200 rpm for 5 min on a ZHWY-103B rotary shaker (Shanghai Zhicheng Analytical Instrument Manufacturing Company, Shanghai, China). The NPs-bacteria conjugates were isolated by applying an external magnet. Then the supernatant was carefully transferred to measure its A_{600}. The capture efficiencies of PDA@Fe_3O_4 NPs for *S. aureus* were calculated based on the decrease of A_{600} relative to a reference before magnetic capture. The NPs-bacteria conjugates were rinsed twice using PBS (10 mM, pH 7.0) and isolated for subsequent use.

2.5. Magnetic extraction of DNA with PDA@Fe_3O_4 NPs

The bacterial DNA was isolated from the captured *S. aureus* by PDA@Fe_3O_4 NPs in two steps. The first step was to lyse bacteria. The NPs-bacteria conjugates was first added to the lysozyme solution (100 μL, 20 mg mL^{-1}) and the suspension was incubated at 37 °C for 1 h. Then, 400 μL lysis buffer (20 mM NaAc, 40 mM Tris-CH_3COOH, 1% SDS, 1 mM EDTA, pH 7.8) was added to the suspension. The tube was incubated at 37 °C for 1 h. Next, 3 μL RNase A solution (10 mg mL^{-1}) was added and the mixture was heated at 37 °C for 15 min. After magnetic separation, the supernatant was transferred to a new 1.5-mL tube. Then, 200 μL of NaCl (5 M) was transferred to the tube. The mixture was centrifuged at 10,000 rpm and 4 °C for 20 min. Afterwards, the cell lysates in supernatant was moved to a new 1.5-mL tube and stored at 4 °C before use.

The second step was to magnetically extract DNA with PDA@Fe_3O_4 NPs. Firstly, an aliquot (300 μL) of cell lysates was added to the binding solution (660 μL, 10 mM MES-HCl, pH 2.0). Then 40 μL PDA@Fe_3O_4 NPs suspension (25 mg mL^{-1}) was added to the tube. The tube was incubated at room temperature for 10 min with gentle agitation. Next, the DNA-PDA@Fe_3O_4 NPs conjugates were collected through magnetic separation, followed by washing twice with ethanol solution (70%,