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2-Step lectin-magnetic separation (LMS) strategy combined with AuNPs-based colorimetric system for *S. aureus* detection in blood

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ABSTRACT

A novel 2-step lectin-magnetic separation (LMS) method combined with gold nanoparticle (AuNPs) -based colorimetric system for specifically and quantitatively detecting *S. aureus* in blood samples was established. In this study, wheat germ agglutinin lectin from *Triticum vulgaris* (wheat) (WGA) was applied for the first time as a recognition agent in a 2-step magnetic separation method using magnetic nanoparticles (MNPs) for effective and low-cost bacteria enrichment. A quantitative detection strategy based on the long dsDNA amplicons stabilized with AuNPs against salt-induced aggregation was investigated. The 2-step LMS method resulted in a high enrichment efficiency even at low concentrations (10^0–10^2 CFU/mL) of *S. aureus* in pure culture and blood samples. The AuNPs-based colorimetric system was applied for quantitative detection of *S. aureus* with a limit of detection (LOD) at 3.5×10^0 CFU/mL. The complete process starting with the blood samples treatment, 2-step LMS, and AuNPs-based colorimetric detection took only 7 h. The low cost and sensitive 2-step LMS combined with AuNPs-based colorimetric detection holds considerable potential for early sepsis diagnosis.

1. Introduction

Sepsis, triggered by infection which overcomes the body's tissues and organs to respond and recover, is a life-threatening condition that challenges clinical diagnosis and treatment [1,2]. Annually, 18 million people gets infected with sepsis and more than 5 million died [3]. *S. aureus* was identified as one of the main etiologic agents of sepsis, but the lack of its early diagnosis has hampered the treatment of sepsis. *S. aureus* is a gram-positive bacterium and has been identified as one of the three most frequent nosocomial pathogens [4]. More seriously, overuse and misuse of antibiotics led to the appearance of bacterial strains that are resistant to multiple types of antibiotics which exacerbated the timely and effective diagnosis and treatment of sepsis caused by *S. aureus* [5]. These made the development of methods for the effective and rapid detection of *S. aureus* in the bloodstream an urgent task.

Broth-based blood culture has been considered as the "gold standard" for the clinical diagnosis of sepsis [6,7] because it is a sensitive approach. However, this process requires skilled peresonnel, as well as labor-intensive requiring several days to obtain the results. Immunological assay provides a more rapid approach but is limited by sensitivity [8]. Polymerase chain reaction (PCR) is more sensitive and specific but require time-consuming and complicated gel electrophoresis-based detection [9]. The disadvantages of the complicated blood matrix that include interferences from the complex blood, the unsatisfactory sensitivity of the immunoassays, and the tedious detection processes involved in the molecular methods have restricted their applications for practical diagnosis of sepsis [10].

Magnetic separation is an inexpensive and efficient method that could be used to solve the interferences from blood and the unsatisfactory sensitivity of the methods used for early diagnosis of sepsis [11,12]. Over the last 20 years, immunomagnetic separation (IMS) has been considered one of the most important strategies in the concentration and isolation of target components in a complex matrix [13,14]. IMS has been used to separate and concentrate whole pathogens, proteins, DNA, and other components from a complex matrix effectively and has been used to eliminate the inhibitory substances in PCR or immunoassays [15,16]. However, traditional IMS has been limited by expensive reagents [17], immunogenic abnormalities caused by mutations [11], and a low enrichment factor [18]. To reduce the costs and minimize the disadvantages presented by immunogenic separation, more economical, convenient, and more efficient recognition agents were studied. Additionally, a 2-step recognition reaction for large volume was proposed to reduce the consumption of expensive recognition agents and to improve the enrichment factor [19].

Wheat germ agglutinin lectin from *Triticum vulgaris* (wheat) (WGA)

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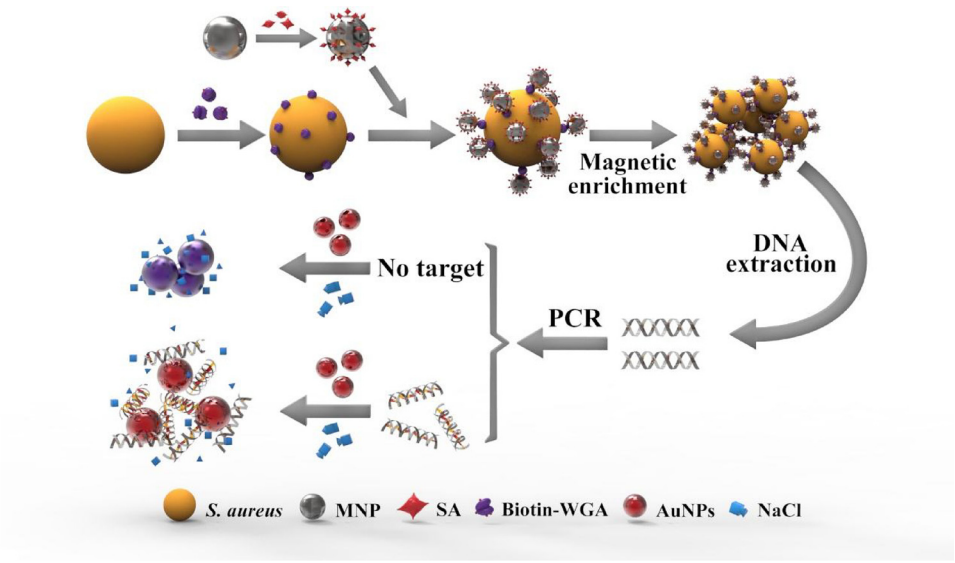

Scheme 1. Schematic diagram for 2-step LMS strategy based on WGA combined with AuNPs-based colorimetric system for *S. aureus* detection in blood.

is a dimer lectin that could be uses as a new recognition agent [20,21]. WGA could specifically recognize *N*-acetylglucosamine and its derivatives through hydrophobic interactions, electrostatic interactions, and hydrogen bonding [22,23]. When applied to bacteria recognition, because of the abundant carbohydrates present on the surface of some bacteria, WGA has been shown to exhibit a strong affinity binding to a broad spectrum of bacteria, which makes it have a very wide application prospect in simultaneous detection of multiple bacteria [21,24]. In addition, WGA is potentially a good candidate for pathogen capture because of its low cost, high stability and availability [25].

Gold nanoparticles (AuNPs) have been widely used in nucleic acid detection because of their colorimetric properties [26–28] in rapid, simple and cost-effective systems [29,30]. Previous reports suggested that AuNPs exhibited greater resistance to color change under the absorption of single- and double-strained DNA (ssDNA and dsDNA) [31–34]. These studies used short fragments of dsDNA (5–50 bp) for detection. In this paper, we report a 2-step large-volume lectin-magnetic separation (LMS) using WGA combined with AuNPs-based colorimetric detection of *S. aureus* in blood. Biotin-WGA were used to capture *S. aureus* through the formation of biotin-WGA ~ *S. aureus* complex which was magnetically isolated through the interaction between biotin and streptavidin (SA) forming MNPs-SA ~ biotin-WGA ~ *S. aureus*. The genomic DNA of *S. aureus* were extracted to obtain sufficient dsDNA amplicons by a traditional PCR. The dsDNA amplicons were detected using adsorption on unmodified AuNP. In this method, the low concentration of bacteria in blood could be detected faster at low cost.

2. Experimental section

2.1. Materials and reagents

Carboxylated MNPs with a 180 nm diameter (10 mg/mL) were purchased from Allrun Nano Science & Technology Co. Ltd. (Shanghai, China). *N*-Hydroxysuccinimide sodium salt (NHSS) and 1-(3-(dimethylamino) propyl)-3-ethylcarbo diimide hydrochloride (EDC) was obtained from Aladdin Industrial Corporation (Shanghai, China). Bovine serum albumin (BSA) was bought from Biofroxx GmbH (Hessen, Germany). Biotin conjugated wheat germ agglutinin lectin from *Triticum vulgaris* (wheat) (biotin-WGA), Tris, glycogen, Triton-X100, ammonium acetate, Na_2EDTA, trisodium citrate ($C_6H_5Na_3O_7{\cdot}2H_2O$), and hydrogen tetrachloroaurate trihydrate ($HAuCl_4{\cdot}3H_2O$) were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Recombinant Proteinase K (20 mg/ml) was obtained from Sangon Biotech Co. Ltd. (Shanghai, China). Luria – Bertani (LB) broth and Baird-Parker Agar Base was bought from Land Bridge Technology Co. Ltd. (Beijing, China). Streptavidin (SA) was purchased from Shanghai hualan Chemical Technology Co. Ltd. (Shanghai, China). Red blood cell lysis buffer was purchased from Solarbio Co. Ltd. (Beijing, China). Lysozyme and BacteriaGen DNA Kit were bought from ComWin Biotech Co. Ltd. (Beijing, China). 2 × Taq Master Mix was obtained from novoprotein Technology Ltd. (Shanghai, China). TaKaRa MiniBest DNA Fragment Purification Kit were purchased from TaKaRa Biotech Co. Ltd. (Dalian, China). PCR primers were synthesized from Tsingke Biological Technology (Wuhan, China).

2.2. Apparatus

The Zeta Sizer Nano ZS90 (Malvern Instruments Ltd., Britain) was used to confirm the presence of SA on the MNPs. The anchoring state of MNPs-SA complex and *S. aureus* was characterized using Scanning electronic microscopy (SEM) (JSM-6701F, JEOL Ltd., Tokyo, Japan) and Laser Scanning Confocal Microscope (LSCM) (Leica Microsystems GmbH, Wetzlar, Germany). Multimode microplate reader (Varioskan LUX, Thermo-Fisher Scientific, Waltham, USA) was used to scan the UV–vis absorption spectrum of AuNPs. Gel electrophoresis photographs were obtained in the presence of a UV transilluminator (ImageQuant LAS 500, GE Healthcare, New York, USA).

2.3. Bacterial culture

Bacterial strains used in this study, including 2 strains of *S. aureus* and 6 non-target strains, were listed in Table S1. All bacterial strains were cultured in LB broth overnight at 37 °C at 180 rpm. The count of bacterial strains was determined with plate count: 0.1 mL of the appropriate dilutions were streaked onto the LB agar that were incubated at 37 °C for 12–24 h, and the colonies grown on the plates were counted to establish the bacteria concentration.

2.4. Preparation of MNPs-SA

The MNPs-SA was prepared following our previous publication [12,14]. Briefly, 6.6 mg NHSS and 5.8 mg EDC was added to 20 mg carboxylated MNPs in 10 mL of sterilized PBS (0.01 M, pH 7.4), and then mixed with a rotator at 15 rpm for 1 h. after washing three times with sterilized PBS (0.01 M, pH 7.4) to remove excess reagents, 1.6 mg