Sensors and Actuators B 270 (2018) 72–79

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

journal homepage: www.elsevier.com/locate/snb

Highly sensitive detection of high-risk bacterial pathogens using SERS-based lateral flow assay strips

Rui Wang[a], Kihyun Kim[a], Namhyun Choi[a], Xiaokun Wang[a], Jiyoung Lee[a], Jun Ho Jeon[b], Gi-eun Rhie[b], Jaebum Choo[a,*]

[a] Department of Bionano Technology, Hanyang University, Ansan, 15588, South Korea
[b] Division of High-risk Pathogens, Laboratory Control of Infectious Diseases, Korea Centers for Disease Control and Prevention, Chungju, 28159, South Korea

ARTICLE INFO

Article history:
Received 29 January 2018
Received in revised form 28 March 2018
Accepted 27 April 2018
Available online 4 May 2018

Keywords:
Yersinia pestis
Francisella tularensis
Bacillus anthracis
Surface-enhanced Raman scattering
Lateral flow assay

ABSTRACT

Bacterial pathogens such as *Yersinia pestis, Francisella tularensis,* and *Bacillus anthracis* are classified into the highest rank of potential bioterrorism agents. Colorimetric lateral flow assay (LFA) strips are commercially available but these conventional strips have drawbacks in terms of low sensitivity and limit of quantitative analysis. Therefore, there is an urgent need for a new sensing platform to detect these pathogens in the early contamination stage. In this study, a novel surface-enhanced Raman scattering (SERS)-based LFA strip was developed for sensitive detection of bacterial pathogens. Target-specific SERS nanotags (Raman reporter-labeled gold nanoparticles) were used as an alternative to the gold nanoparticles in conventional LFA strips. Using these SERS nanotags the presence of bacteria could be identified through a simple color change in the test line. Additionally, highly sensitive and accurate quantitative analysis could be performed by monitoring the characteristic Raman peak intensity of SERS nanotags that were captured in the test line. This highly sensitive method required a short assay time (15 min) and a tiny volume of pathogen sample (40 μL). We believe that the proposed SERS-based LFA technique has great potential as a valuable tool in the early detection of specific bacterial pathogens in the field due to its excellent analytical sensitivity.

1. Introduction

High-risk bacterial pathogens such as *Yersinia pestis, Francisella tularensis*, and *Bacillus anthracis* are categorized as lethal infectious agents that can be used as biological weapons for bioterrorism [1–3]. The Center for Disease Control and Prevention in the United States has classified them as "Category A" bio-threat agents, the highest rank among potential bioterrorism agents.

Y. pestis is a Gram-negative coccobacillus that causes an acute and lethal infection in humans and animals [4–6]. *Y. pestis* is notorious for its use as a biological weapon in World War II. The primary carriers of this pathogen are the Oriental rat flea and infected rodents [7]. *Tularemia*, also known as rabbit fever, is a serious infectious zoonotic disease caused by the bacterium *F. tularensis*, which is a non-motile, non-spore forming, Gram-negative, rod-shaped coccobacillus [8,9]. Inhalation of aerosolized bacteria and ingestion of contaminated food and water lead to pneumonic tularemia [10]. Anthrax is caused by *B. anthracis*, a Gram-positive and endospore forming bacterium. The spread of anthrax is closely associated with contact with bacterial spores, which are composed of dehydrated cells with thick walls and additional layers that form inside the cell membrane [11]. Since these spores are highly resilient, surviving in extreme temperatures, low-nutrient environments, and under harsh chemical treatment, *B. anthracis* has been considered one of the most dangerous bioterrorism agents [12,13]. Infection of humans with these highly contagious agents causes high morbidity and mortality and subsequently leads to widespread panic and social disruption, which is an extremely serious threat to national security. Therefore, it is critical to develop a rapid and sensitive detection technique for these high-risk bacterial pathogens.

Conventionally, bacteria colony counting has been considered the gold standard in the detection of *Y. pestis, F. tularensis,* and *B. anthracis* but it needs a long culture time up to several days because of the pre-enrichment and selective differential plating steps [14,15]. Real-time polymerase chain reaction (RT-PCR) is also extensively used as a microbiological identification technique, but it also needs sample pretreatment steps including DNA extraction and signal amplification by a sequential thermo-cycling process

* Corresponding author.
E-mail address: jbchoo@hanyang.ac.kr (J. Choo).

https://doi.org/10.1016/j.snb.2018.04.162
0925-4005/

[16,17]. In particular, any erroneous amplification of unrelated gene sequences leads to incorrect identification in RT-PCR. Therefore, there is still an urgent demand for a new detection platform that allows rapid and reliable detection of these bacterial pathogens [18,19].

Recently, a lateral flow assay (LFA) strip has attracted increasing attention as an alternative tool for the detection of high-risk pathogens because of its many advantages including a user-friendly format, fast detection time, and long-term stability [20–24]. LFA strips for three high-risk bacterial pathogens are commercially available but a more sensitive and accurate sensing platform is absolutely needed to reduce the risk posed by microorganisms as biological weapons [25,26] since the current commercial LFA strips possess major limitations in terms of detection sensitivity and limit of quantification capability. To improve these two factors, we developed a surface-enhanced Raman scattering (SERS)-based bacteria LFA sensor for the first time. We recently utilized the SERS-based LFA platform for sensitive analysis of staphylococcal enterotoxin B [27], HIV-1 DNA [28] and thyroid-stimulating hormone [29] but there has been no report about high-risk bacterial pathogens till now. Since the size of bacterial pathogen is much bigger than the size of protein, DNA or hormone, experimental conditions of running buffers and SERS nanotags should be carefully optimized. Here, we report the application of SERS-based LFA sensor for the highly accurate and sensitive analysis of low-abundance high-risk bacterial pathogens. This approach also provides new insights into early detection of specific bacterial pathogens in the field.

When reporter molecules are adsorbed onto the surface of gold nanoparticles, their Raman signals are greatly enhanced at SERS active sites known as “hot spots” as a result of electromagnetic and chemical enhancement effects [30–32]. In the SERS-based LFA assay platform, Raman reporter-labeled SERS nanotags were used as a detection probe and the presence of a target pathogen could be identified by the naked eye. In addition, highly sensitive quantitative analysis can be performed with the aid of a Raman reading system. In this study, a novel SERS-based LFA platform was proposed for highly sensitive detection of three high-risk bacterial pathogens. Target-specific SERS nanotags (Raman reporter-labeled gold nanoparticles) were used as an alternative to the gold nanoparticles utilized in conventional LFA strips. Highly sensitive quantitative evaluation of bacterial pathogens could be achieved by monitoring a characteristic Raman peak intensity change of SERS nanotags on the test line. This approach has strong potential to be a feasible method for on-site early detection of high-risk bacterial pathogens due to its excellent analytical sensitivity.

2. Experimental section

2.1. Reagents and materials

Gold (III) chloride trihydrate ($HAuCl_4{\cdot}3H_2O$), tris-sodium citrate (Na_3-citrate), dihydrolipoic acid (DHLA), 1-ethyl,3-(3-dimethylaminopropyl)carbodiimide (EDC), 4-(4-maleimidophenyl)butyric acid *N*-succinimidyl ester (NHS), bovine serum albumin (BSA), polyvinylpyrrolidone (PVP), Tween 20, Borax (pH 9.0), and Tris-EDTA (pH 8.0) were purchased from Sigma-Aldrich (MO, USA). Surfactant 10G was purchased from Fitzgerald (CA, U.S.A.). Malachite green isothiocyanate (MGITC) and phosphate-buffered saline (PBS, 10×, pH 7.4) were purchased from Invitrogen Corporation (CA, USA). Inactivated *Y. pestis, F. tularensis*, and *B. anthracis* were supplied by the Korea Center for Disease Control and Prevention (KCDCP). *Y. pestis* antibody sets were also provided by KCDCP. Mouse monoclonal antibody sets against *F. tularensis* (T14 and FB11) and against *B. anthracis* spores (SA26 and SA27) were purchased from HyTest (Turku, Finland). The nitrocellulose (NC) membrane attached to a backing card (HF090 MC100) was purchased from Millipore Corporation (MA, USA), and the absorbent pad (CF4) was purchased from Whatman-GE Healthcare (PA, USA).

2.2. Preparation of antibody-conjugated SERS nanotags

Gold nanoparticles (AuNPs) were prepared using the previously reported seeded-growth method [33]. All glassware was washed using aqua regia, rinsed with distilled water, and oven-dried prior to use. A 75-mL solution of 2.2 mM sodium citrate was heated to boiling, and 0.5 mL of 25 mM $HAuCl_4$ was added to the flask upon boiling. The color of the solution changed from light yellow to bluish gray and then to soft pink in 15 min. The resulting gold seed solution was cooled to 90 °C. To this solution, 0.5 mL of 60 mM sodium citrate and 0.5 mL of 25 mM $HAuCl_4$ solution were sequentially added 12 times at 2-min intervals to ensure complete mixing after each addition, and the color of the solution finally changed from pink to deep red. The solution was stirred for a further 30 min at 90 °C and then was cooled to room temperature. The shape and size distribution of AuNPs was characterized by dynamic light scattering (DLS) and transmission electron microscopy (TEM).

SERS nanotags were prepared using the method previously reported. Briefly, 1 μL of 10^{-4} M MGITC (Raman reporter) was added to 1.0 mL of AuNP solution and reacted for 30 min under vigorous shaking. For conjugation of antibodies on the surface of AuNPs, their colloid solution was controlled at pH 9 by addition of 100 μL of 0.1 M borax buffer solution. And then 4 μL of 1 mg/mL antibody was added to the MGITC-labeled AuNPs solution. After shaking for 2 h at room temperature, 20 μL of 10% BSA was added to block the unbound surfaces of AuNPs. The mixture was shaken for 30 min and centrifuged at 6000 rpm for 10 min to remove non-specific binding chemicals and antibodies. After discarding the supernatant, the pellets were re-dispersed in buffer solution.

2.3. Preparation of LFA strips

The typical LFA strip is composed of four compartments: a sample pad, a conjugate pad, a NC membrane with plastic backing card, and an absorbent pad. First, a test line and a control line were marked on the NC membrane by dispensing 0.5 mg/mL of capture antibody and 0.1 mg/mL of secondary antibody that binds the detection antibody on SERS nanotags, respectively. Each antibody was dispensed on the NC membrane at a rate of 0.8 μL/cm using a precision line dispensing system (Zeta Corporation, South Korea). The dispensed membranes were dried for 1 h at room temperature. The assembly membrane was cut into 3.8 mm-wide strips using an automatic paper-cutting instrument (Zeta Corporation). To simplify the operation procedure, the LFA strip marked with test and control lines was directly dipped into wells of a 96-well ELISA plate containing a sample solution and SERS nanotags as a dipping substrate.

2.4. Instrumentation

UV-vis absorption spectra were collected with a Cary 100 spectrometer (Varian, Salt Lake City, UT, USA). Dynamic light scattering (DLS) measurement was performed with a Nano-ZS90 instrument (Malvern, UK) and TEM images were acquired using a JEOL JEM 2100F instrument at an accelerating voltage of 200 kV. Enzyme-linked immunosorbent assay (ELISA) was performed using a microplate reader (Power Wave X340, Bio-Tek, Winooski, VT, USA) equipped with a 96-well plate. Raman spectra for the test line of the LFA strip were acquired using a Renishaw InVia Raman microscope system (Renishaw, New Mills, UK); a He–Ne laser with a power of 20 mW operating at λ = 633 nm was utilized as the exci-