Biosensors and Bioelectronics 72 (2015) 230–236

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Fast and sensitive detection of an anthrax biomarker using SERS-based solenoid microfluidic sensor

Rongke Gao [a,1], Juhui Ko [a,1], Kiweon Cha [b], Jun Ho Jeon [b], Gi-eun Rhie [b], Jonghoon Choi [a], Andrew J. deMello [c,*], Jaebum Choo [a,*]

[a] Department of Bionano Technology, Hanyang University, Ansan 426-791, South Korea
[b] Division of High-Risk Pathogen Research, Center for Infectious Diseases, National Institute of Health, Cheongju 363-951, South Korea
[c] Department of Chemistry and Applied Biosciences, Institute of Chemical and Bioengineering, ETH Zürich, Vladimir Prelog Weg 1, 8093 Zürich, Switzerland

ARTICLE INFO

Article history:
Received 3 March 2015
Received in revised form
30 April 2015
Accepted 4 May 2015
Available online 6 May 2015

Keywords:
Anthrax
Surface-enhanced Raman scattering
Microfluidics
Solenoid
Poly-γ-D-glutamic acid

ABSTRACT

We report the application of a fully automated surface-enhanced Raman scattering (SERS)-based solenoid-embedded microfluidic device to the quantitative and sensitive detection of anthrax biomarker poly-γ-D-glutamic acid (PGA) in solution. Analysis is based on the competitive reaction between PGA and PGA-conjugated gold nanoparticles with anti-PGA-immobilized magnetic beads within a microfluidic environment. Magnetic immunocomplexes are trapped by yoke-type solenoids embedded within the device, and their SERS signals were directly measured and analyzed. To improve the acccuracy of measurement process, external standard values for PGA-free serum were also measured through use of a control channel. This additional measurement greatly improves the reliability of the assay by minimizing the influence of extraneous experimental variables. The limit of detection (LOD) of PGA in serum, determined by our SERS-based microfluidic sensor, is estimated to be 100 pg/mL. We believe that the defined method represents a valuable analytical tool for the detection of anthrax-related aqueous samples.

1. Introduction

Anthrax is an acute disease caused by the gram-positive bacterium *Bacillus anthracis* (Little and Ivins, 1999; Turk, 2007; Boyden and Dietrich, 2006; Park et al., 2002). Infection occurs only when spores enter the body by inhalation or consumption of contaminated food or water. If the spores are active, bacteria multiply, spread, produce toxins and ultimately cause severe illnesses. Over the past two decades, the potential use of anthrax as a biological weapon by terrorists has become an increasingly significant threat. For this reason, the Center for Disease Control and Prevention of the United States classifies *B. anthracis* as a Tier 1 select agent with high bioterrorism potential (Skyberg, 2014). Accordingly, rapid and sensitive methods for the detection of anthrax *in vivo* are urgently needed for both early diagnosis and successful treatment after exposure. Recently, it has been reported that the *B. anthracis* capsule is composed of poly-γ-D-glutamic acid (PGA), which is closely associated with the pathogenesis of the *B. anthracis* infection since it protects bacilli from immune surveillance (Scorpio et al., 2007; Jang et al., 2013). Consequently, the PGA capsule can be used as a target marker for the detection of *B. anthracis*.

Various detection methods, such as immunofluorescence microscopy (Dal Molin et al., 2006; Zornetta et al., 2010), enzyme-linked immunoassays (ELISA) (Dominguez-Castillo et al., 2012; Seo et al., 2015) and polymerase chain reaction (PCR)-based assays (Euler et al., 2013; Seiner et al., 2013) have been used for the direct identification of *B. anthracis*. Unfortunately, these methods have significant technical drawbacks such as poor limits of detection, extensive sample pretreatment and unacceptably long assay times, which make them undesirable for both laboratory and in-the-field implementation. Accordingly, there is a demonstrated and pressing need for rapid, direct and sensitive detection of *B. anthracis* in humans.

Surface-enhanced Raman scattering (SERS) detection is an emerging read-out technique that allows the sensitive, selective and fast detection of biomarker molecules (Porter et al., 2008; Driskell et al., 2005; Li et al., 2008; Han et al., 2008). For this reason, the application of SERS-based techniques for fast and sensitive disease diagnosis has become increasingly popular. To date, variety of biomarkers has been quantitatively probed using SERS-based immunoassays. One of the most popular SERS assay platforms is based on a sandwich immunoassay containing

* Corresponding authors. Fax: +82 31 436 8188.
E-mail addresses: andrew.demello@chem.ethz.ch (A.J. deMello), jbchoo@hanyang.ac.kr (J. Choo).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.bios.2015.05.005
0956-5663/

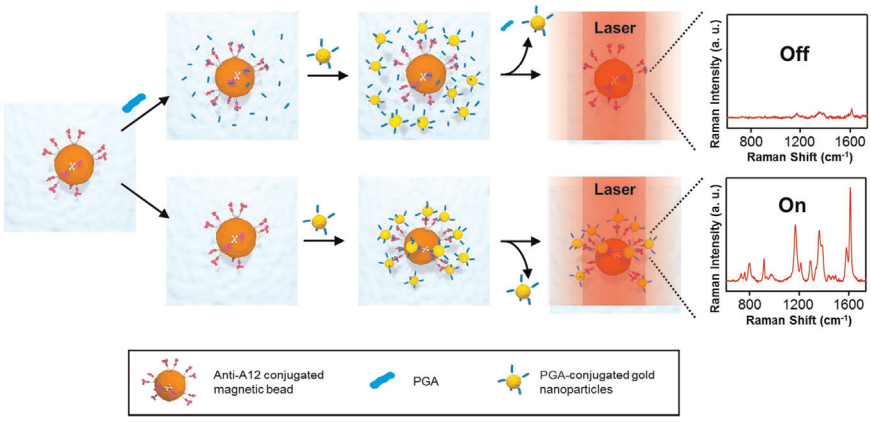

Fig. 1. Schematic illustration of the SERS-based competitive immunoassay for quantification of PGA marker in serum. PGA-conjugated AuNPs will not bind on anti-A12 conjugated magnetic bead in the presence of PGA, resulting in a weak SERS intensity (top). In contrast, strong SERS signals are observed without PGA (bottom).

primary antibody-conjugated magnetic beads (as substrates) and secondary antibody-conjugated SERS nano-tags (as probes) (Lee et al., 2011; Gong et al., 2007; Chon et al., 2009, 2011). Here, antigen is sandwiched between the two antibodies, with the binding affinity between the antibody and antigen defining assay sensitivity. Unfortunately, this sandwich assay protocol suffers from a cross reactivity and long assay time (Porter et al., 2008; Chon et al., 2009).

To resolve these issues, SERS-based competitive immunoassays have been developed (Chon et al., 2014). For example, we recently reported the feasibility of SERS-based competitive immunoassay using anti-PGA conjugated magnetic beads PGA antigen-conjugated gold nanoparticles (AuNPs) for the fast and reproducible detection of PGA markers (Ko et al., 2015). A schematic illustration of the SERS-based competitive immunoassay process for PGA is shown in Fig. 1. AuNPs conjugated with PGA antigens are utilized as "SERS nano-tags" and anti-PGA-immobilized magnetic beads as supporting substrates. When free PGA target antigens and PGA-conjugated AuNPs are mixed with magnetic beads, they undergo competitive reaction with the antibodies on magnetic beads. After the reaction is complete, immunocomplexes are isolated by a magnetic bar, and then unreacted free PGA target antigens and PGA-conjugated AuNPs removed. The SERS signals of the immunocomplexes are then measured. As more PGA antigens is added, the surface loading of bound PGA increases, and less PGA-conjugated AuNPs remain on the magnetic beads, resulting in decrease of SERS intensity of immunocomplexes.

Nonetheless, such SERS-based competitive immunoassays using conventional microtubes and magnetic bars raise technical issues: such as an inhomogeneous distribution of magnetic immunocomplexes on the wall of a microtube, the requirement for manual washing steps and magnetic field intensities. To address these drawbacks, we herein implement a competitive immunoassay into a solenoid-embedded dual-channel microfluidic device containing both sensing and control channels, for the rapid immunoassay of PGA trace markers in human serum. This proposed method is expected to be a useful analytical tool for the fast detection of anthrax in solution.

2. Experimental

2.1. Materials and reagents

Gold (III) chloride trihydrate (> 99.9%), sodium citrate dehydrate (99%), bovine serum albumin (BSA), ethanolamine, Rhodamine 6G (R6G), Rhodamine B (RB), dihydrolopic acid (DHLA), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and N-hydroxysuccinimide (NHS) were purchased from Sigma-Aldrich, and used without further purification. Poly-γ-D-glutamic acid (PGA) and A-12 antibodies were supplied by the Korea Centers for Disease Control and Prevention. Biotin-PEG 5000-NHS, used for the bio-functionalization of capture magnetic beads, was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Streptavidin-coated magnetic beads and malachite green isothiocyanate (MGITC) were purchased from Invitrogen Corporation (Carlsbad, CA, USA). The average diameter of the magnetic beads was 1 μm. SeraSub® synthetic serum was obtained from CST Technologies. Phosphate-buffered saline (PBS) solutions containing 0.05% Tween-20 (v/v) at pH 7.4 were prepared using standard methods. Polydimethylsiloxane (PDMS, Sylgard 184 Silicone. Elastomer Kit) was purchased from Dow Corning. Deionized water was purified using a Milli-Q water purification system (Millipore Corporation, Billerica, MA, USA).

2.2. Preparation of PGA-conjugated gold nanoparticles (AuNPs)

AuNPs were synthesized using the citrate-reduction method reported by Frens (1972). In brief, 100 mL of 0.01% gold chloride trihydrate solution was heated to boiling, and 1.0 mL of 1% trisodium citrate dehydrate solution added under vigorous stirring. Within a few seconds, the color of the solution changed from faintly blue to brilliant red, indicating the formation of AuNPs. After the mixture was boiled for 20 min, heat was removed and the solution was stirred for 1 h. TEM images and size distributions determined by the dynamic light scattering measurements have been displayed in Fig. S1. AuNPs show homogeneous size distribution, and the average particle size is estimated to be 41.5 ± 3.5 nm. To prepare SERS-active nanoprobes, 0.5 μL of 0.1 mM MGITC was added to 1.0 mL of 0.1 nM AuNPs, and the mixture was reacted for 1 h under stirring. A 2.5 μL of 0.1 mM DHLA was first added into 1 mL of Au NPs solution. After incubation for 1 h, 2.5 μL of 0.1 mM EDC and NHS were added and allowed to react with the activated the –COOH terminal groups of the DHLA molecules for 15 min. Finally, 1.0 μL of 1 mg/mL PGA was added to the NHS-activated AuNPs and reacted for 1 h. Unreacted NHS groups on the surface of the AuNPs were deactivated by adding 2.5 μL of 0.1 mM ethanolamine for 20 min. Nonspecific binding chemicals were removed through centrifugation, and the remaining nanoprobes were washed with PBS buffer solution three times.

2.3. Preparation of PGA antibody-conjugated magnetic beads

For the bioconjugation of magnetic beads, 0.1 mL of Biotin-PEG