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High performance immunochromatographic assay combined with surface enhanced Raman spectroscopy☆

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ABSTRACT

We present a novel biosensor concept achieved by integrating a widely used lateral-flow immunochromatography (LF-ICA) system with a highly sensitive surface enhanced Raman spectroscopy (SERS) technique to overcome the lack of sensitivity of the conventional LF-ICA that employs gold nanoparticle (AuNP) as a tracer. The SERS phenomena can be produced from hotspot formation between enlarged AuNPs labeled with Raman reporters by applying a simple silver intensifying process, resulting in a significant Raman signal enhancement. Several major factors that influence the analytical performance of the system such as laser power, integration time, nanoparticle size, and silver intensification time were experimentally optimized to maximize Raman intensity. Consequently, compared with the conventional LF-ICA, this new method shows approximately 1000-fold increase in detection sensitivity compared to the FluB assay, enabling detection of extremely low concentration of the analyte without additional complicated steps.

1. Introduction

Lateral-flow immunochromatographic assay (LF-ICA) in which a porous membrane strip is utilized as the immunosorbent (e.g., antibody) is a simple immunological assay and one of the most practical and widely used methods for the detection of target analytes in sample matrices such as blood and urine [1]. In general, a lateral flow through the immunostrip that consists of different types of membranes expedites the reaction, allowing it to be completed in a relatively short time (less than 15 min) and further enabling the *in situ* separation of unreacted components via a one-step analysis, which is a key advantage of this assay format.

To date most LF-ICA format is intended to operate on a purely qualitative basis, generating color signals from gold nanoparticle tracers that are perceivable by the naked eye but exhibiting sensitivities lower than those associated with the conventional immunological methods, particularly enzyme-linked immunosorbent assay (ELISA) [2,3]. This feature is also definitely insufficient for the assessment of a number of biomarkers where quantitative analyses for early diagnosis and progress monitoring are classically constrained.

Surface-enhanced Raman spectroscopy (SERS) is a spectroscopic technique that exploits the enhancement of Raman scattering by molecular adsorption on rough metal surfaces. This technique enables detection of molecules by their vibrational signatures [4–7]. Two theories are generally used to explain the SERS phenomenon, an electromagnetic mechanism, which postulates an enhanced electromagnetic field at the surface of noble metal nanostructures, the other being a chemical enhancement which calls for an electronic resonance charge transfer between the metal surface and the bonded molecule [8,9]. As visible and NIR wavelengths are used to excite the Raman modes, gold and silver are considered for recording the SERS signals since their surface plasmon resonance frequencies correspond to that range [10]. Due to the high amplification factor, SERS has been applied for highly sensitive detection of biomolecules, down to single molecule level [5].

In this study, we have shown the high performance of LF-ICA in combination with the advantages of SERS technique assisted by silver intensification for the detection of FluB which was used as a model analyte. The silver intensifying method, also known as autometallography, was applied to produce SERS signals caused by hotspot formation on the surface of gold nanoparticles (AuNPs).

☆ Work was completed at Purdue University and the first author is now at Eulji University.

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2. Materials and methods

2.1. Materials

CS18187 and CS18185 FluB antibodies were kindly supplied by Meridian Bioscience (Cincinnati, OH). Bovine serum albumin (BSA), hydrogen tetrachloroaurate ($HAuCl_4$), sodium citrate, 4-mercaptobenzoic acid (MBA), tannic acid, 3,3′,5,5′-tetramethylbenzidine (TMB), silver enhancer solution A and B and casein were purchased from Sigma–Aldrich (St. Louis, MO). The silver enhancers A and B were mixed in the ratio of 1 to 1 and then applied (50 μL) to the absorption pad in cross-flow direction for signal amplification. Horseradish peroxidase, anti-BSA monoclonal antibody, streptavidin-HRP and goat anti-mouse IgG coupled to HRP were obtained from Pierce (Rockford, IL). Other reagents used were of analytical grade.

2.2. Preparation of SERS probes

Probe I: 1 mL of AuNP solution synthesized via citrate reduction [11] (30 nm in diameter) was first mixed with 1 μL of 0.5 M of Na_2CO_3, 100 μL of phosphate buffer (10 mM, pH 7.4) and 10 μg of CS18187 Flu B antibody solution, which was stirred in a revolver for 2 h. 10% (w/v) BSA dissolved in 10 mM phosphate buffer (122 μL) was added and then stirred for 1 h. 30 μL of 1 mMMBA solution dispersed in absolute ethanol is added and reacted further for 2 h. Unbound molecules were removed by centrifugation (12,000 rpm, 15 min) and the pellet was resuspended using 1 mL of 0.5% (w/v) casein in phosphate buffer (casein-PBS), and this step was repeated three times. The pellet was finally resuspended in 100 μL of casein-PBS and stored at 4 °C.

Probe II: AuNP with a mean diameter of 3 nm was synthesized using tannic acid and sodium citrate as the reducing agents [12]. 10 μg of anti-BSA antibody dissolved in 10 mM phosphate buffer was reacted with 1 mL of gold solution under gentle shaking for 2 h. 30 μL of 1 mM MBA was added and further reacted for 2 h. Residual surface of the conjugate was blocked with casein-PBS at room temperature for 1 h. The solution was centrifuged at 14,000 rpm for 45 min and the pellet was re-suspended in 1 mL of casein-PBS, which was repeated three times. The pellet was finally resuspended in the same solution. The product was then stored at 4 °C.

2.3. Probe analysis

For immunoassay of probe I, 100 ng of FluB antigen (1 μg/mL) dissolved in PBS (10 mM phosphate containing 150 mM NaCl, pH 7.4) was coated on the microwell at 37 °C for 1 h. This incubation condition was identically applied to the following reaction steps. The residual surface was blocked with 200 μL of casein-PBS and 100 μL of probe I diluted in the casein-PBS solution was then reacted. For verification of antibody and BSA attachment to the gold surfaces, goat anti-mouse IgG coupled to horseradish peroxidase (HRP) and biotinylated mouse anti-BSA antibody followed by reacting with streptavidin-HRP were respectively reacted. For signal generation 200 μL of substrate solution (50 mM sodium acetate: 1% (w/v) TMB: 3% (v/v) hydrogen peroxide = 1000:10:1) was added and maintained for 15 min. After halting the reaction by adding 50 μL of 2 M sulfuric acid, the optical density was measured by ELISA reader (VERSAmax; Molecular Device, Sunnyvale, CA) at 450 nm. For verification of probe II, the whole procedure was identical to probe I assay except that BSA was coated on the microwell plate.

2.4. Analytical procedure of LF-ICA assay

0.5 μg of CS18185 Flu B antibody was coated on the nitrocellulose membrane by dropping the antibody solution and the membrane was dried at 37 °C for 30 min. To expedite the immunoassay on the membrane strip, Probes I and II were placed on the corresponding conjugate pad followed by FluB antigen diluted in casein-PBS (100 μL) application to the sample absorption pad. After 15 min of letting the sample flow for immunoreaction to occur, the strip was washed with 40 μL of deionized water by connecting two absorption pads on the other side of nitrocellulose membrane for inducing cross-flow. For silver intensification, two silver enhancer solutions mixed in 1:1 ratio was applied to the absorption pad and then washed with deionized water to the stop reaction. The strip was dried and then subjected to Raman measurement.

2.5. Transmission electron microscopy analysis

Transmission electron microscopy (TEM) images of the SERS probes were obtained using a Tecnai T20™ transmission electron microscope (FEI™, Hillsboro, OR). The two freshly prepared probe samples (before and after silver intensification process) were drop-casted directly on formvar/carbon 400 mesh TEM grid and then air-dried before taking TEM measurements.

2.6. Raman spectroscopic measurements

A SENTERRA™ confocal Raman system (Bruker Optics, Billerica, MA) fitted with a 785 nm laser, and a 50× air objective (N.A. 0.7) was employed for spectroscopic and imaging measurements. Integration time (20 s), laser power (10 mW), size of gold nanoparticle used and the allowable reaction time for silver staining were varied and the conditions optimized for SERS measurement. To get normalized spectrum, the background signals were subtracted from the original spectrum using the accompanied software

3. Results and discussion

3.1. Analytical concept

Our goal is to develop an integrated biosensor combining the simple interface of LF-ICA with the high sensitivity of surface enhanced Raman spectroscopy (SERS) to detect low amount of target analyte in a sample. To this end, SERS tags can be incorporated at the surface of AuNP which provides a way to characterize the surface property from the known spectrum of the Raman reporter molecule. In the present application, 4-mercaptobenzoic acid (MBA) was considered as a Raman reporter of choice as it has a relatively large Raman cross section and well-characterized spectrum peaks [13]. The overall scheme of the assay is depicted in Fig. 1. For making two types of probes (probe I and probe II), two different sizes of AuNPs (30 and 3 nm in diameter) were used. 30 nm AuNP was functionalized with an antibody specific to the target antigen, MBA and BSA for probe I and 3 nm AuNP was modified with anti-BSA antibody and MBA for probe II. These probes were then placed on the corresponding region of the immunostrip.

As the sample is applied to the strip (a), the target analyte migrates and binds to the probe I via antigen–antibody interaction (b), which was further bound to probe II on which the anti-BSA antibody was coated through its interaction with BSA on the probe I (c). The resulting reagents were then moved to the signal generation area of the nitrocellulose membrane where the capture antibody was pre-immobilized to result in a sandwich immunocomplex formation (d). To enlarge the size of the two probes, treatment with a silver enhancer solution was done by supplying the reagent in a different flow direction, i.e., cross-flow (e). Gold provides an electron transfer route from the reducing agent such as hydroquinone to silver ions bound to the gold surface facilitating specific deposition of metallic silver at the site of immunogold labeling [14]. To