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Sensitive immunosensor for respiratory syncytial virus based on dual signal amplification of gold nanopaticle layer-modified plates and catalyzed reporter deposition

Lei Zhan [a], Tong Yang [a], Chun Mei Li [a], Wen Bi Wu [a], Cheng Zhi Huang [a,b,*]

[a] Key Laboratory on Luminescence and Real-Time Analytical Chemistry (Southwest University), Ministry of Education, College of Pharmaceutical Sciences, Southwest University, Chongqing 400715, China
[b] Chongqing Key Laboratory of Biomedical Analysis, Chongqing Science & Technology Commission, College of Chemistry and Chemical Engineering, Southwest University, Chongqing 400715, China

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ABSTRACT

It is still a challenge to identify and control the outbreak of infectious diseases at their earliest stages because of the lack of ultrasensitive, rapid, and selective diagnostic technologies. We have developed and describe here a highly sensitive immuno-biosensor for the fast and unambiguous detection of respiratory syncytial virus (RSV) by accompanying catalyzed reporter deposition with gold nanoparticle layer-modified plate which can significantly amplify the binding density of capture antibody. The captured RSV pulls down the horseradish peroxidase (HRP)-labeled detection antibody through sandwiched immunoreactions on the plate, where the HRP drives the deposition and accumulation of biotinalyated-tyramide molecules in the presence of hydrogen peroxide, in turn increasing the load of streptavidin-HRP by the biotin-streptavidin interaction. As a result, the developed immunosensor displays high sensitivity for the detection of RSV with a dynamic range from 0.05 pg/mL to 30 pg/mL and the lowest detectable concentration of 0.01 pg/mL. Intra-assay and interassay coefficients of variation are 2.63% and 5.52%, respectively. Furthermore, it can be adapted to the detection of RSV spiked in complex samples, showing good accordance with the conventional ELISA method.

1. Introduction

The development of novel bioassays for accurate, sensitive, and rapid pathogen detection is of paramount important for timely clinical decision-making and management of epidemics of infectious diseases [1]. Immunoassay based on specific antigen-antibody recognition has been recognized as one of the most widely used tools for pathogen detection due to its great reliability and excellent accuracy [2]. So far, many kinds of immunoassays such as enzyme-linked immunosorbent assay (ELISA) [3,4], electrochemistry [5,6], chemiluminescence [7,8], fluorescence [9,10], surface enhanced Raman scattering (SERS) [11,12], and surface plasmon resonance (SPR) [13,14] immunoassay have been developed. Typically, ELISA has attracted a great deal of attention since it provides several advantages as compared to other immunoassays such as low cost, ease of fabrication, and rapid/direct readout with the naked eye [15]. However, the conventional ELISA is unable to monitor the pathogen in most settings where the level of pathogen is generally very low. To tackle this issue, a variety of strategies for signal amplification have been incorporated into ELISA such as polymerase chain reaction [16], DNA hybridization chain reaction [17], silver enhancement reaction [18] and nanomaterials including nanoparticles [19] and liposome [20] to improve the detection sensitivity. In particular, gold nanoparticle (GNPs), one of the most extensively studied nanomaterials, have great potential for use in immunoassay because of their unique optical, electrical, chemical, and catalytic properties [21]. Ambrosi et al. developed an enhanced GNPs-based ELISA for the analysis of CA 15-3 antigen, an important breast cancer biomarker present in blood samples [22]. This approach adopting GNPs as multienzyme carrier results in higher sensitivity and shorter assay time when compared to classical ELISA procedure. In addition, Liu et al. reported a sandwich type immunoassay for sensing ultralow levels of prostate-specific antigen (PSA) in patient serum samples using GNPs-based fluorescence-activatable probe, where

* Corresponding author at: Key Laboratory on Luminescence and Real-Time Analytical Chemistry (Southwest University), Ministry of Education, College of Pharmaceutical Sciences, Southwest University, Chongqing 400715, China.
E-mail address: chengzhi@swu.edu.cn (C.Z. Huang).

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GNPs are employed as highly efficient fluorescence quenchers [23]. Recently, Liu et al. presented a novel plasmonic ELISA that allows the ultrasensitive detection of enterovirus 71 (EV71) using acetylcholinesterase (AChE) as enzyme label which catalyzes its substrate acetylthiocholine to generate thiocholine, inducing the aggregation of GNPs and further resulting a colour change of GNPs solution from red to purple, which can be observed by the naked eye [24]. In the above immunoassay formats, GNPs can be efficiently conjugated to proteins such as antibody or enzyme while maintaining their biological activities, and served as excellent nano carrier, quencher, or signal readout for signal amplification process.

On the other hand, enzyme-mediated signal amplification strategy such as catalyzed reporter deposition (CARD) system has been easily implemented not only in immunohistochemistry but also in situ hybridization protocol [25]. The method, also called tyramide signal amplification (TSA), takes advantage of the reaction between horseradish peroxidase (HRP) and tyramide [26]. In the presence of H_2O_2, HRP oxidizes the phenolic rings of tyramide to produce a short-lived and highly reactive phenolic radical, which bind preferentially with electron-rich amino acid residues of proteins around the enzyme. Generally, tyramide with conjugates (e.g., biotin, fluorescein, cyanine dyes, etc.) is used in TSA system because the covalently bound reporter labels can be further detected either by avidin-biotin-enzyme complex visualization technique or by fluorescence microscopy [27]. Recently, Akama et al. described a droplet-free digital enzyme-linked immunosorbent assay based on a tyramide signal amplification system, permitting the highly sensitive detection of protein biomarkes [28].

Encouraged by the high loading capability of GNPs and the great signal enhanced ability of CARD system, herein we prepared a stable gold nanoparticle layer (GNPL)-modified plate and developed a highly sensitive immunoassay system for the detection of respiratory syncytial virus (RSV) in complex samples. RSV has been recognized as one of the leading causes of severe respiratory disease burden in young children, immunocompromised patients and elderly individuals, resulting enormous economic losses [29]. Moreover, RSV infection fails to induce protective immunological memory, thus repeated infections can often be seen throughout life [30]. Up to now, there is not yet a convincing and safe vaccine available [31]. Hence, rapid and sensitive diagnosis of RSV infection is extremely important to implement infection control and guide antiviral therapy.

2. Experimental

2.1. Materials and apparatus

The HEp-2 epithelial cell line was obtained from Xiangya Hospital of Central South University (Hunan, China). RSV strain was purchased from Guangzhou Bote Biological Technology Development Co., Ltd. (Guangzhou, China). Both anti-RSV antibody (ab20745) and HRP-labeled anti-RSV antibody (ab20686) were purchased from Abcam Inc. (Cambridge, MA). Hydrogen tetrachloroaurate (III) tetrahydrate ($HAuCl_4 \bullet 4H_2O$) was purchased from Sinopharm Group Chemical Reagent Co., Ltd (Shanghai, China). 3, 3′, 5, 5′-Tetramethylbenzidine (TMB) was purchased from Aladdin Industrial Corporation (Shanghai, China). Hydrogen peroxide (30 wt%, H_2O_2) and glucose were obtained from Chuandong Chemical (Group) Co., Ltd. (Chongqing, China). Bovine serum albumin (BSA) and common 96-well microplates were purchased from Beijing Dingguo Changsheng Biotechnology Co., Ltd. (Beijing, China). Other reagents were of analytical grade and used as received. The TSA kit was purchased from PerkinElmer Life Sciences (Boston, MA). The kit comprised a biotinylated-tyramide (B-T) solution, amplification diluents concentrate and streptavidin-HRP (SA-HRP) concentrate. Enhanced bicinchoninic acid (BCA) protein assay kit was the product of Beyotime Biotechnology (China). Ultrapure water obtained from a Millipore water purification system (≥18 MΩ, Milli-Q, Millipore) was used in all assays. 0.1 M carbonate/bicarbonate buffer (pH 9.6) and 0.1 M phosphate buffered saline consisted of 0.137 M NaCl and 0.003 M KCl (pH 7.4) were prepared. The spectrophotometric measurements were performed using an Epoch microplate spectrophotometer (Biotek, USA). Scanning electron micrographs were taken using a Hitachi S-4800 scanning electron microscope (Tokyo, Japan).

2.2. Preparation of GNPL-modified plate

Gold nanoparticle layer was fabricated according to the previous literature with a little modification [32]. Briefly, the solution for chemical gold plating containing 12 mM $HAuCl_4$, 25 mM glucose, and 0.5 M $NaHCO_3$ was prepared prior to use. Here, glucose serves as not only an reducing agent for gold ions but also an capping agent to stabilize the gold nanoparticles, while $NaHCO_3$ is used to control the pH of the reaction solution. Then various volumes (50 μL, 100 μL, 150 μL, 200 μL, 250 μL) of plating solution were injected into each well of a 96-well microplate. Meanwhile, a certain volume of ultrapure water was introduced until the total volume of each well was 250 μL. The plate was then kept at 37 °C for 3 h to form GNPL (a), GNPL (b), GNPL (c), GNPL (d), and GNPL (e), respectively. Afterwards, the as-prepared GNPL-modified plate was rinsed with ultrapure water three times and dried at room temperature for further use.

2.3. Porosity analysis

Porosity of GNPL (a–e) was analyzed via SEM images using *ImageJ* software, a public domain Java-based program that was originally developed at the National Institutes of Health. The SEM images should be first converted to binary images by selecting an appropriate threshold value. Then the area of the pores has been calculated using the built-in "Analyze Particle" function on the ImageJ program menu by fitting the pores with ellipses. It should be noted that all images are at the same magnification. Finally, the porosity which refers to the analyzed pores relative to the total area of image can be calculated.

2.4. Protein adsorption assay

Human serum albumin (HSA), HRP, and IgG were selected for the protein adsorption sudy. The GNPL-modified wells were first incubated with 100 μL of protein solutions (1 mg/mL) at 37 °C for 2 h. Subsequently, the excess proteins were collected and the concentrations were quantified using a BCA protein assay according to manufacturer's instructions. Then the amount of the adsorbed proteins on the GNPL substrate can be calculated.

2.5. HRP activity assay

An aliquot of 100 μL of 1 mg/mL HRP was added to the wells modified with GNPL and incubated at 37 °C for 2 h. The wells were rinsed three times to remove the unbound enzyme. Then TMB enzyme substrate (100 μL) was added to each well, and the plates were incubated at 37 °C for 15 min. Color development was stopped by additiong of 2 M sulfuric acid (50 μL). The optical density was read at 450 nm with a microplate reader within 15 min after stopping the reaction. The activity of the same amount of free enzymes was determined by the same procedure as described.