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Magneto-plamonic nanoparticles enhanced surface plasmon resonance TB sensor based on recombinant gold binding antibody

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ABSTRACT

The magneto-plamonic nanoparticles (MPNs) have been employed to enhance the signal of surface plasmon resonance (SPR) spectroscopy to gain new insight into MPN and gold film SPR interaction. Three different morphologies MPNs (sphere, short spiky and long spiky) of Fe_3O_4@Au nanoparticles with good dispersion were synthesized and characterized carefully. A sandwich SPR immunosensor was constructed by immobilizing gold binding anti-CFP-10 (Ab1) onto a gold chip surface via Au-S bond firstly. Gold binding anti-CFP-10 (Ab2) captured on MPN surface was utilized to amplify the SPR signals specifically. Compared with spiky MPNs, the structure of spherical MPN, which concentrates the electric charge density and immobilize more Abs on its surface coverage, could enhance the electronic coupling effect significantly. Attribute to the super paramagnetism of MPNs, a facile solution route was fabricated to capture and separate analyte from the real sample by outside extend magnetic field. Implementation of MPNs results in 30-fold enlargement of the SPR signal at the limit of detection. To this end, an immunoassay is carried out that couples the specificity of antibody-antigen interactions with the high sensitivity based on spherical MPNs signal enhancement SPR.

1. Introduction

Tuberculosis (TB), a common infectious disease caused by *Mycobacterium tuberculosis* (Mtb), has been described since ancient times and its causative agent. According to WHO, an estimated 2 million deaths and more than 9 million new cases annually caused by TB. Due to its high efficient infectivity through atmosphere, TB is out of control in developing countries. In Southeast Asia and sub-Saharan Africa, where the infection rate reaches 700 cases per 100,000 individuals [1], which makes TB continues to be one of the most important infectious causes of death worldwide [2]. Despite mass *Mycobacterium* vaccination and the rapid development of anti-tubercular drugs, the diagnosis of TB still remains a challenge toward global public health problem.

Nowadays, a number of diagnostic methods have been applied in the clinic. The classical treatment protocols are long-lasting [3]. Traditional methods for the diagnosis of TB include clinical radiography observation, microscopic detection of TB in sputum samples stained with specific dyes, culture of Mtb from patient samples and the purified protein derivative (PPD) tuberculin test. However, the efficacy of sputum smear microscopy test is low in the case of HIV-positive patients and children, and the reliability of this test is heavily dependent on the expertise of the microscopist performing the test [4–6]. Culture-based diagnostic methods improve the sensitivity and specificity of TB diagnosis, and allow an assessment of drug susceptibility that can only be determined by growing Mtb in culture; however, these methods can take as long as 6 weeks to achieve diagnosis and fail to reach a diagnosis in approximately 50% of cases [7–9]. More advanced culture-based systems, for example, the mycobacteria growth indicator tube, offer relative improvements in sensitivity and speed over traditional techniques by combining, Mtb culture and monitoring in 1 tube. However, this technique still requires more than 10 days of culturing time and an expensive fluorescence monitoring system for Mtb detection [10].

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Thus, there is a desperately clinical need for a simple, economical and easily performed method for the rapid and effective diagnosis of TB especially for developing countries. In order to meet the increasing demand for sensitive detection of TB, a signal amplification strategy with nanomaterials is used in this work based on TB marker protein in the tissue fluids from TB patients. Targets for antigen detection must be highly immunogenic and abundantly secreted in culture medium. Among Mtb antigens, CFP-10 protein is an early secretary antigen and also an abundant antigen of the culture filtrate of Mtb [11]. This protein is not present in *Mycobacterium bovis* BCG or most non-tuberculosis mycobacteria [12] and is used as an antigen for diagnosis based on interferon stimulation [13,14].

Surface plasmon resonance (SPR) sensing has been demonstrated in the past decade to be an exceedingly powerful and quantitative probe of biomolecular interactions. It provides a mean not only for identifying these interactions and quantifying their equilibrium constants, kinetic constants and underlying energetics, but also for employing them in very sensitive, label-free biochemical assays. However, SPR technique suffers the low sensitivity to small change in refractive index especially for the small molecules. The modifications of the biosensors for enhancing sensitivity of SPR have been fabricated, mainly through the efficient immobilization of receptor and the introduction of nanomaterials for signal enhancement. Among them, the application of gold nanoparticles (GNPs) [15–19] and magnetic nanoparticles (MNPs) [20–22] in SPR optical biosensor have been attracted increasing attention. Lyon et al. has applied GNPs as the signal-amplifying label in the sandwich structure immunoassay ascribed to its biocompatibility and high index of refraction [23]. SPR biosensors fabricated with MNPs have been applied in the separation and detection of bioactive compounds [20,22]. The super paramagnetism of MNPs is also used to accelerate the reaction process, resulting in the improvement of sensor's sensitivity [20]. Magneto-plamonic nanoparticles (MPNs) have recently received much attention due to their unique optical and electrochemical properties, which make them potential materials in various fields. Typically, MNPs are composed of gold and magnetic nanomaterials, where magnetic nanomaterials as a core can be viable alternatives to conventional materials for separation supports. Moreover, the combination of Au nanomaterials with a magnetic core not only provides stable binding sites to the biomaterials but can also be a means of signal amplification for SPR, thus offering an ideal platform to study the multifunctionality of nanomaterials.

In this work, a new TB sensor was developed based on the commercially prism-coupled SPR by improving its immobilization of receptors and amplification of response signals. Recombinant gold binding antibody (anti-CFP-10, Ab1) was immobilized on the gold film simply and abundantly. Second recombinant gold binding antibody (Ab2) was combined with MPNs directly to form MPNs-Ab conjugate. The strong electric coupling between anisotropic MPNs and surface plasmon wave effectively promotes the changes in imaginary components of the refractive index of thin film at the chip/solution interface. Formation of conjugate also leads to change of the real component of the refractive index with the increased mass of MPNs-Ab2 conjugate. Synergistic effect of gold binding Ab and multifunctional MPNs-Ab conjugate contribute to the significant sensitivity enhancement of the TB sensor response. Furthermore, the developed TB sensor was evaluated in artificially CFP-10-containing urine. In the presence of the targets, MPNs-Ab conjugate interact with the target and was separated from sample by external magnetic field without complex separation and purification. A sensitive format for immunosorbent assays has been developed to meet the increasing levels of performance demanded in the medical, veterinary, and bioterrorism prevention arenas.

2. Experimental

2.1. Materials and reagents

Bovine serum albumin (BSA) was obtained from Zhaorui Biotech Co., Ltd. (Shanghai, China). Artificial urine was purchased from Huzhou Inno Reagents Co., Ltd. (Zhejiang, China). 10 mM phosphate buffer saline (PBS) and all other reagents were obtained from Sigma (St. Louis, MO). Milli-Q grade (18.2 MΩ cm) water was used for preparation of sample and buffer solutions. All proteins are dissolved in phosphate-buffer saline (PBS, pH 7.4). 0.1 mg mL^{-1} BSA in PBS solution was used for blockage of nonspecific binding.

2.2. Synthesis of core shell gold capped magnetic nanoparticles

Under nitrogen atmospheric, 1.622 g of $FeCl_3{\cdot}6H_2O$ and 0.9941 g of $FeCl_2{\cdot}4H_2O$ was dissolved into 40 mL of ultrapure water with constant mechanical stirring. After the reagents had completely dissolved, 5 mL of ammonia solution (28%, w/v %) was quickly added to the reaction mixture. Ten minutes later, we added 4.4 g of trisodium citrate and the reaction temperature was raised to 90 °C, and maintained at that temperature with continuous stirring for 30 min. A black precipitate was obtained by cooling the reaction mixture to room temperature [24]. The precipitate was then thoroughly rinsed with ethanol for three times. During rinsing, samples were separated from the supernatant using a permanent magnet. After discarding the supernatant, samples were dried under vacuum and stored at 4 °C for further use.

To synthesis spherical MPNs, all the glassware were cleaned with aqua regia (HCl/HNO_3 at a 3:1 ratio by volume) and rinsed with ethanol and ultrapure water (*Caution! Aqua regia is very corrosive oxidizing agent which should be handled carefully*). $HAuCl_4$ (20 mL, 0.5 mM) was heated to boiling and vigorously stirred in a round-bottom flask. Then rapid addition 10 mL of the MNPs solution prepared from the previous step resulted in a continuous color change from brown to burgundy. The ratios of reactive volumes between Fe_3O_4 and $HAuCl_4$ solution were 1:2. Stirring continued for 10 min after the color change ceased [24]. The solution was cooled to room temperature with a continuous stirring for another 45 min. The spherical MPNs were centrifuged three times at 6500 rpm for 30 min. After that, the product was washed with ultrapure water and re-dispersed in ultrapure water.

The spiky Fe_3O_4@Au NPs were synthesized by reducing $HAuCl_4$ with 4-(2-hydroxyethyl)-1-piperazineethane-sulfonic acid (HEPES). An aqueous stock solution of 100 mM HEPES was prepared with ultrapure water. The pH was adjusted to 7.4 by adding 1 M NaOH at 25 °C. Then, 10 mL of 100 mM HEPES (pH 7.4) was mixed with 500 μL of 0.136 μM of the Fe_3O_4@Au NPs solution followed by the addition of 250 μL of 20 mM $HAuCl_4$. Without shaking, the color of the solution changed from light brown to green and finally to yellow-green at room temperature within 30 min. The different morphologies of spiky Fe_3O_4@Au NPs were tuned by altering the concentration of HEPES in the reaction system.

2.3. Preparation of CFP-10

The recombinant CFP-10 protein was expressed in *E. coli* and purified as previously described [25]. Briefly, the corresponding gene was amplified by polymerase chain reaction (PCR) using *M. tuberculosis* genomic DNA as a template and then inserted into pET-28a vector for expression in bacteria. The recombinant plasmid was transformed into *E. coli* BL21 cells. The cells were grown at 37 °C until the optical density at 600 nm was 0.4–0.5 and then induced with 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG). The bacteria were then harvested by centrifugation and lysed by sonication. The recombinant protein was purified from