

DEVELOPING GOLD NANOPARTICLE-BASED BIOSENSORS FOR SIMPLE AND FAST DETECTION OF *STAPHYLOCOCCUS AUREUS*

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ABSTRACT:

Nanoparticles-based biosensors are emerging technologies for detection of pathogens. It is very challenging to develop nanoparticle-based biosensors for whole cells due to the larger size of cells compared with the nanoparticles. In this study, a new gold nanoparticle-based biosensor is developed to detect bacterial cells. Instead of trying to capture bacteria on the solid substrate, in this study, the DNA aptamers, which are capable of binding a target cells, are allowed to detach from the gold nanoparticle surface when interacting with bacteria on the sensor surface. This approach greatly increases the sensitivity of plasmonic biosensor. The results demonstrated that this sensor could detect *S. aureus* with a detection limit of $\sim 10^3$ CFU/ml within 30 min. This method showed many advantages compared with conventional methods with respect to cost, simplicity and analysis time.

Keywords: DNA aptamer, gold nanoparticles, *S. aureus*.

1. Introduction

Staphylococcus aureus is one of 32 species of genus *Staphylococcus* bacteria. Mostly other species only found in mammals and does not infect humans. *S. aureus* was firstly described by the German physician Anton Rosenbach in 1884. They are a non-motile, clustered, globular, Gram-positive, facultative anaerobic bacterium. *S. aureus* cells are approximately 1 μ m and are distinguished from other staphylococcus by its ability to produce a yellow color in culture, positive reactions such as coagulase, mannitol

fermentation, deoxyribonuclease [1]. *Staphylococcus aureus* mostly lives on the skin and mucous membranes of warm-blooded animals. In addition, since nasal membranes are warm and moist, this is an effective habitat for growing of bacteria in nose, for this, 10% - 40% of adults made this disease. Infections caused by *S. aureus* can spread to people through contact with pus from the wound, skin, or objects when individuals are infected. People who have joint replacement surgery are capable of risk of developing necrotizing fasciitis, infection of the

endocardial surface of the heart, and pneumonia when infecting with *S. aureus* [2]. Thus, early diagnosis is exceptionally important for clinical screening, medical management, and disease surveillance at the first onset.

Traditional methods are popular in the testing of pathogenic microorganisms. Fundamentally, steps for *S. aureus* detection following traditional methods are proliferation, isolation, biochemical confirmation and serum screening. *S. aureus* can suffer presence of high salt concentration, just 7.5%, which coagulates rabbit plasma and then produce positive reactions such as coagulase, mannitol fermentation, deoxyribonuclease. *Staphylococcus aureus* can be detected and quantified through its specific unique characteristics in tested patient samples and food. Advantages of traditional methods are sensitivity, quantification of properties of microorganism presence in food samples. However, disadvantages of this method are time-consuming for experiment (3-7 days) and labor-intensive [3-6]. Currently, there are several approaches for the elimination of the time-consuming step of bacterial culture and reduction of the time for analysis such as immunoassays and amplification techniques. Among these, metallic nanoparticles have shown a great potential for detection and quantitation of pathogens because of outstanding optical properties of the noble nanoparticles which relate to the interaction between free electrons of the nanoparticles and the electromagnetic field of the incident light [7]. The application of nanoparticles in biosensors is now very promising due to significant increasing the performance of the biosensor [8].

High-affinity antibodies with analytes are often chosen for immunoassays. However, the use of antibodies has some disadvantages such as instability and high-cost [9]. Recently, a new class of targeting compounds, so-called aptamers have exploited as an alternative substance to antibodies for bioassays. The aptamers are short, single-stranded DNAs/RNAs that have high affinity and they can fold into intricate 3D conformation when binding with a wide range of targets [10].

Compared with immunoassays, aptamer-based biosensors bear many advantages such as high specificity and high stability against biodegradation and denaturation. Herein, we reported an DNA aptamer-based plasmonic biosensor for rapid detection of *S. aureus* with a low cost. The detection method uses DNA aptamer that is specific to *S. aureus* as the recognition element of plasmonic biosensor. Instead of trying to capture *S. aureus* on the solid substrate, our approach allows the DNA aptamers to detach from the Au surface when interacting with bacteria. Using *S. aureus* as a model for the pathogen detection, the plasmonic biosensor could detect $\sim 10^3$ CFU/ml within 30 min. The results indicated that this biosensor could be exploited as a powerful tool for whole-cell detection of pathogens in a short time.

2. Materials and methods

2.1. Materials

Hydrogen tetrachloroaurate (III) trihydrate ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$, 99.9%), (AgNO_3 , 99%), Sodium citrate dihydrate ($\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$), sodium borohydride (NaBH_4 , 98%), sodium chloride (NaCl) and phosphate buffer saline pH 7.4 were purchased from Sigma-Aldrich, Inc. (USA). The DNA aptamer of *S. aureus* (5'-TCC CTA CGG CGC TAA CCT CCC AAC CGC TCC ACC CTG CCT CCG CCT CGC CAC CGT GCT ACA AC-3') [11] was obtained from PHUSA Biochem Co. (Vietnam). The reagents and essential chemicals used in this study were available in AR grade. All glassware was carefully cleaned with a freshly prepared solution of aqua regia and rinsed carefully with ultrapure water before use.

2.2. Bacterial cultures and harvest conditions

S. aureus was cultured in Tryptic Soy Broth (TSB) medium for 16 h. The concentration of *S. aureus* was determined by serial dilution with subsequent plating on agar plates and measurement of colony forming units (CFUs). CFUs were also determined by measuring optical density (OD) at 600 nm (an OD₆₀₀ of $1.0 \approx 1.5 \times 10^9$ CFU/ml). The bacteria were got by centrifugation at $1,000 \times g$ (3785 rpm for rotor radius 6.25 cm) for 15 min at 4°C, washed three

times with physiological saline, and finally suspended in physiological saline for further use.

2.3. Synthesis of Oval-Shaped Gold Nanoparticles

In this study, oval shape AuNPs with aspect ratio of 1:3 were synthesized using seed-mediated growth procedure as described by Wentong et al with minor modification [12]. In brief, The gold seeds were synthesized by adding 2 mL of 0.5 mM HAuCl₄ to 2 mL of 0.2 M cetyltrimethylammonium bromide (CTAB), followed by vigorous stirring. To the stirred solution, 240 μ L of the freshly prepared ice-cold 0.01 M NaBH₄ was added, which resulted in the formation of the brownish yellow seed solution which was stirred vigorously for 30 s. Next, the solution was gently stirred for an additional 15 min at 45°C to remove excess NaBH₄. The prepared gold seed was used within 1–3 h after its preparation. For preparation of oval-shaped gold nanoparticles, 4.75 mL of 0.0085 M CTAB solution was added in a small vial, and then 0.2 mL of 0.01 M HAuCl₄ was injected into the vial under constant stirring. After that, we used 0.03 mL of 0.01 M AgNO₃ dropwise to allow the solution to mix properly. Once the solution was mixed properly, we added 0.032 mL of 0.1 M ascorbic acid slowly, as a reducing agent. The solution turned colorless. To this colorless solution, 0.01 mL of gold-seed was added at a time and gently mixed the solution for 30 s. Color of solution changed immediately and became dark blue within 2 min. The solution was then filtered by a 0.22 μ m filter to remove any aggregated nanoparticles. The morphology of oval shaped AuNPs was analyzed by UV-vis spectrophotometer and scanning electron microscopy.

2.4. Preparation of APTES coated glass slides

The APTES coated glass slides were prepared for Au nanoparticles immobilization as mentioned in the previous report [13]. The microscopic slide (22 $\times$ 40 $\times$ 0.1 mm) from Warner Instruments was cleaned by the freshly prepared aqua regia solution or piranha solution for 15 min followed by rinsing the slides thoroughly with DI water. Then, the cleaned microscopic slide was dried with nitrogen

gas, and was dipped in 5% (v/v) APTES in 99.9% ethanol for 15 min. After that, the slide was sonicated in DI water 3 times for 5 min. After the washing step, the silanisation was finalized by drying at 120°C for 2 h.

2.5. Preparation of plasmonic aptasensor

Oval shape AuNP solution was sonicated (30 s) for even distribution in the stock solution before dropping. A total of 50 μ L of the gold nanoparticle solution was dropped on an aminosilane coated glass slide and incubated for 1 h at 4°C in a humidity chamber. Unbound AuNPs were washed off by rinsing the slide in distilled water. 50 μ L of PolyLysine (PLL, 70k Da M.W., 0.1% in distilled water) was added onto the AuNP-immobilized glass and were incubated at room temperature for 5 min in the humidity chamber. Unbound PLL was washed off by rinsing the slide in distilled water. Aptamers at 500 nM were fully dehybridized and stretched by heating to 90°C for 2 min in a dry block heater (HeatBlock I, VWR International, LLC, West Chester, PA) above a calculated melting temperature of 64°C. A 50 μ L aliquot of the aptamer solution was added onto the PLL coated AuNP-immobilized glass and incubated for 1 h in the humidity chamber. Unbound aptamers were gently washed off by rinsing the slide in PBS by gently pipetting.

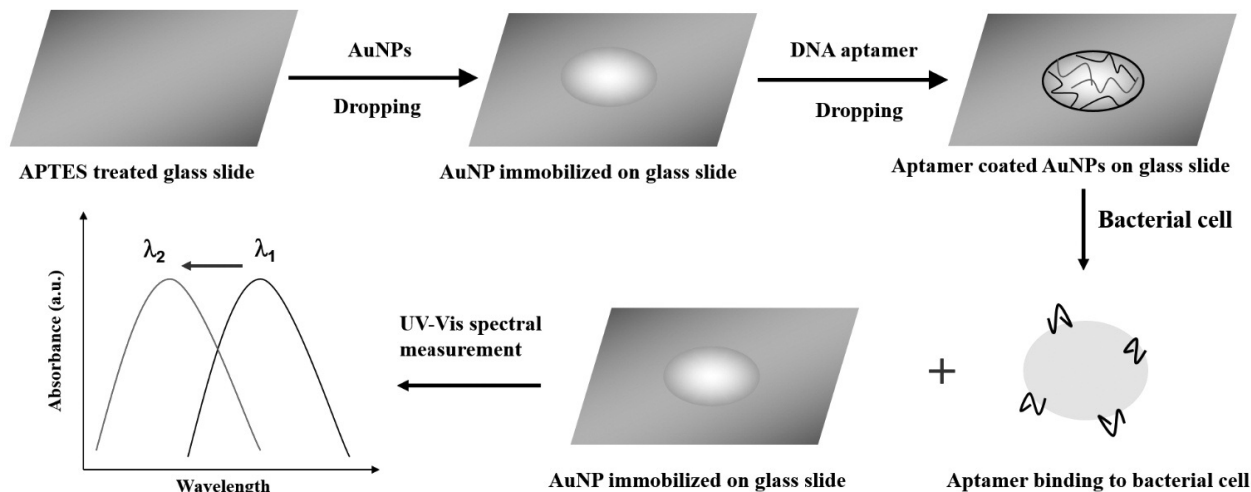
2.6. Detection of *S. aureus* using nanoplasmonic aptasensor

For assay, 50 μ L of various concentrations of *S. aureus* ATCC 25923 from 100 to 10⁸ CFU/ml were dropped at DNA aptamer coated AuNP-immobilized glass and incubated for 20 min at room temperature (~25°C) in the humidity chamber. Target-combined aptamers and unbound molecules were washed off by gently pipetting each biosensor with 50 μ L of PBS while avoiding cross-contamination. The response of biosensor was evaluated by UV-vis spectral measurement. The sensitivity and selectivity of the aptasensor for detection of *S. aureus* was evaluated by exposing the sensor surface to *S. epidermidis*, which is the close relative with *S. aureus*.

3. Results and discussion

The proposed sensing method is based on the

Figure 1. A schematic procedure depicting the experimental processes of the aptamer-based plasmonic biosensor for detection of *S. aureus*



adsorption of the single-stranded DNA(ssDNA) on the gold nanoparticles immobilized on APTES treated glass slide. In this case, ssDNA is able to uncoil its bases to make them exposed to the nanoparticles surface. The electrostatic interaction between the negative charge of ssDNA and the positive charge of polyLlysine-coated gold nanoparticles helps attach the ssDNA on the nanoparticle surface. In the proposed method, we used DNA aptamer as a specific recognition factor and polyLlysine-coated gold nanoparticles (PLL coated AgNPs) as a signal transducer. As displayed in Figure 1, the glass slide was first treated with 3-Aminopropyltriethoxysilane (APTES). The thiol (-SH) group APTES were utilized to conjugate with AuNPs via Au-S covalent bonding. After immobilization of AuNPs on glass, the Au nanoparticles were incubated with negatively-charged DNA aptamers, which is specific to *Staphylococcus aureus*, and the AuNP-DNA aptamer hybrid formed based on the electrostatic interaction. After that the plasmonic biosensor was incubated with *S. aureus* cell suspension. Since the binding bacterial cell - DNA aptamer is much stronger than the electrostatic force between the DNA aptamer and AuNPs, DNA aptamers were released from the AuNPs, inducing a change in local refractive index [14,15]. The shift in LSPR peak, which corresponds to the concentration of

bacterial cells in the sample, was recorded by a miniaturized UV-Vis spectroscopy.

In this study, oval-shaped AuNPs were synthesized using a seed-mediated growth procedure in the presence of positively charged CTAB [12]. This method yielded dark blue coloured aqueous dispersion of oval shape AuNPs with aspect ratio of 1:3. This oval-shaped AuNPs has only one resonance peak like spherical AuNPs, but their max values shifted to higher wavelength, in comparison to the spherical AuNPs of the same size. The size of oval shape AuNPs was about 50 nm, as revealed by UV/Vis spectrophotometer and scanning electron microscopy (SEM). The oval shape nanoparticles were fairly homogenous in shape, and the wavelength of surface plasmon resonance (SPR) occurred at 535 nm (Figure 2). For such a LSPR-shift-based biosensor, it is expected to have a larger peak shift for a small amount of target analytes. In this case, the oval shape AuNPs offers maximal sensitivity to local refractive index change, which subsequently results in better optical response of biosensors [12].

Figure 3 shows the UV-Vis spectrums measured at different steps. The AuNPs immobilized on glass substrate had a SPR peak at 536 nm. The peak of LSPR shifted to 550.5 nm after conjugating with the DNA aptamers. After incubation with *S. aureus* 10^6

Figure 2. (A) Photograph depicting visual color of synthesized oval-shaped AuNPs solution. (B) UV-Vis spectrum of citrate capped silver nanoparticles. (C) Morphology of synthesized oval shaped AuNPs (SEM)

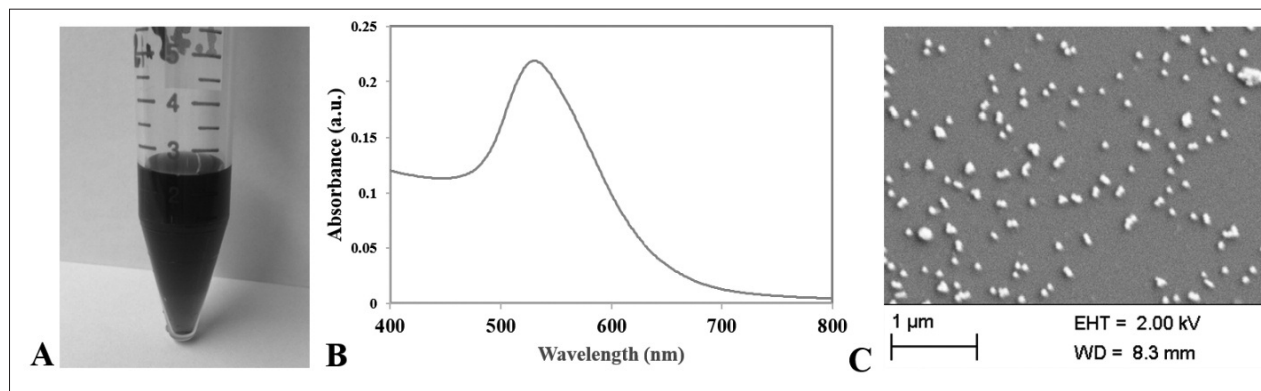
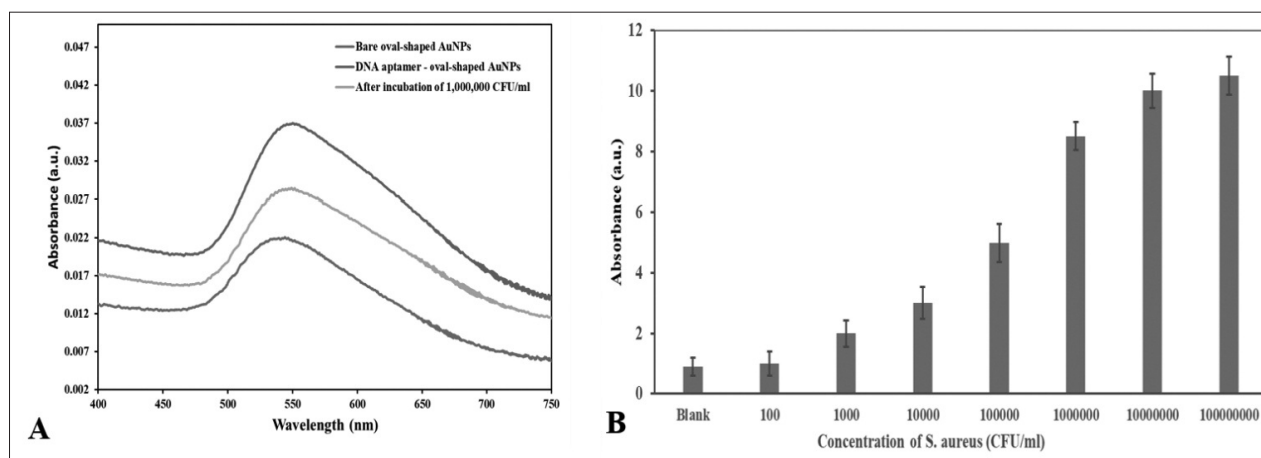


Figure 3. (A) UV-Vis spectrums of oval shaped AuNPs immobilized on glass slide measured at different steps. (B) The signal response of plasmonic biosensor for detection of *S. aureus* at concentrations ranging from 0 to 108 CFU/ml



CFU/ml, the peak moved back to 539 nm, indicating the aptamers detached from the AuNPs. To investigate the sensitivity, 100 μ l of *S. aureus* suspension with different concentrations was introduced to the plasmonic biosensor. Figure 4 shows the detection limit of 10^3 cfu/ml, comparable to that achieved by solution-based biosensing [12]. The detection limit of this sensor can be comparable to the SPR based biosensor with the same bacteria (10^4 CFU/ml) [17]. Compared to the limit of detection based nucleic acid amplification assays for detection of *S. aureus* such as PCR (<10 CFU/reaction) [18], our proposed biosensor has poorer the limit of detection. However, the proposed method takes 30 min for the direct

detection of the whole bacteria without specialized instrumentation and pretreatment steps such as DNA extraction. In comparison with traditional methods, this biosensor can detect *S. aureus* in the real sample with a total analytical time of a few hours.

4. Conclusions

In this study, we have developed a new AuNPs-aptamer-based assay which provides portable, fast, and sensitive biosensors with simple operation based on the principle that bacteria and AuNPs bind competitively to DNA aptamer. The plasmonic biosensor can be used as a powerful analytical tool for detection of whole cell in field of clinical diagnostics and food safety ■

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CẢM BIẾN SINH HỌC DỰA TRÊN NANO VÀNG ĐỂ PHÁT HIỆN NHANH, ĐƠN GIẢN TỤ CẦU KHUẨN *STAPHYLOCOCCUS AUREUS*

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TÓM TẮT:

Cảm biến sinh học nano là công nghệ mới nổi dùng để phát hiện nhanh mầm bệnh. Tuy nhiên, rất khó phát triển cảm biến sinh học nano để phát hiện tế bào, do kích cỡ lớn của tế bào so với hạt nano. Trong nghiên cứu này, tác giả phát triển cảm biến sinh học nano mới để phát hiện tế bào vi khuẩn. Thay vì cố gắng bắt vi khuẩn trên bề mặt cảm biến sinh học nano, cách tiếp cận cho phép DNA aptamer, là chất có khả năng liên kết với tế bào đích, tách khỏi trên bề mặt cảm biến sinh học nano khi tiếp xúc với tế bào vi khuẩn. Cách tiếp cận này cho phép tăng độ nhạy phát hiện của cảm biến sinh học. Kết quả cho thấy loại cảm biến này có thể phát hiện tụ cầu khuẩn *S. aureus* với giới hạn phát hiện khoảng 10^3 CFU/ml trong 30 phút. Phương pháp này có nhiều ưu điểm so với phương pháp truyền thống như tính đơn giản, chi phí và thời gian phân tích.

Từ khóa: DNA aptamer, nano vàng, tụ cầu khuẩn *S. aureus*.