Biosensors and Bioelectronics ■ (■■■■) ■■■–■■■

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

A fluorescence resonance energy transfer (FRET) biosensor based on graphene quantum dots (GQDs) and gold nanoparticles (AuNPs) for the detection of mecA gene sequence of *Staphylococcus aureus*

Jingyu Shi [a], Chunyu Chan [a], Yukting Pang [a], Weiwei Ye [a], Feng Tian [a], Jing Lyu [a], Yu Zhang [b], Mo Yang [a,*]

[a] Interdisciplinary Division of Biomedical Engineering, The Hong Kong Polytechnic University, Hung Hom, Kowloon, Hong Kong, China
[b] Department of Mechanical Engineering, The Hong Kong Polytechnic University, Hung Hom, Kowloon, Hong Kong, China

ARTICLE INFO

Article history:
Received 6 June 2014
Received in revised form
22 September 2014
Accepted 22 September 2014

Keywords:
Graphene quantum dots (GQDs)
Gold nanoparticles (AuNPs)
Fluorescence resonance energy transfer (FRET)
Foodborne bacteria

ABSTRACT

In this work, a novel fluorescence resonance energy transfer (FRET) biosensor based on graphene quantum dots (GQDs) and gold nanoparticles (AuNPs) pairs was developed for *Staphylococcus aureus* specific gene sequence detection. This FRET biosensor platform was realized by immobilization of capture probes on GQDs and conjugation of reporter probes on AuNPs. Target oligos then co-hybridized with capture probes and reporter probes to form a sandwich structure which brought GQDs and AuNPs to close proximity to trigger FRET effect. The fluorescence signals before and after addition of targets were measured and the fluorescence quenching efficiency could reach around 87% with 100 nM target oligo. The limit of detection (LOD) of this FRET biosensor was around 1 nM for *S.aureus* gene detection. Experiments with both single-base mismatched oligos and double-base mismatched oligos demonstrated the good sequence selectivity of this FRET biosensor.

1. Introduction

Staphylococcus aureus (*S. aureus*) is a kind of notorious foodborne bacterium with the ability to produce heat-resistant toxins in food (Le Loir et al., 2003). *S. aureus* has become the second major bacteria in food poisoning, which threatens the health of both human and animals (Soriano et al., 2002). According to the USDA's economic research service, the infectious foodborne illness caused by *Staphylococcus* food poisoning is over 180 thousand cases annually in US (Leonard et al., 2003). Therefore, rapid and efficient detection of *S. aureus* is essential for providing the best treatment to the infected patients.

The conventional method for detecting *S. aureus* is primarily based on bacteria isolation, which is sensitive and inexpensive but bears the limitation of time-consuming and labor-intensive procedures (Carbonnelle et al., 2007; Xiao et al., 2007). Immunological assay such as enzyme-linked immunoabsorbent assay (ELISA) is a rapid method but suffers from relatively low sensitivity (Freed et al., 1982; Yazdankhah et al., 1998). Nucleic acid based assays such as polymerase chain reaction (PCR) have shown advantages of high sensitivity and high specificity for *S. aureus* detection (Carroll et al., 1996). However, the PCR method suffers from expensive equipment, complicated procedures and the need for skillful technicians.

Over the last decade, biosensing techniques have been used for rapid and sensitive foodborne bacteria detection (Alocilja and Radke, 2003; Yang et al., 2004; Yu et al., 2009; Chan et al., 2013). Among them, biosensors based on the hybridization between target DNA and capture DNA enables direct, sensitive, and rapid detection of bacterial DNA without target amplification (Pang et al., 2013). Various sensing mechanisms are developed for DNA detection such as quartz crystal microbalance (QCM) (Mao et al., 2006) and surface plasmon resonance (SPR) (Wang et al., 2011) and electrochemical biosensor (Wang et al., 2009; Luo et al., 2013). Fluorescence resonance energy transfer (FRET), which can transmit photo excitation energy from a donor fluorophore to an acceptor fluorophore, is a technique widely used in biosensing field (Storhoff et al., 2004; Gore et al., 2014). Traditional fluorophores include FAM, Texas red, and Cy5, which are limited by high cost and photobleaching effect. Therefore, they are not suitable for reliable and long term detection. In most recent years, graphene quantum dot (GQD) has received considerable attentions as fluorescence labels in biosensing due to its high brightness, long fluorescence lifetime, and good photo-stability (Zhang and Wang, 2012; Dong et al., 2012; Sun et al., 2013; Qian et al., 2014). GQD can be excited with a short-wavelength light source, usually in the UV

* Corresponding author. Fax: +852 2334 2429.
E-mail address: Mo.Yang@polyu.edu.hk (M. Yang).

http://dx.doi.org/10.1016/j.bios.2014.09.059
0956-5663/

region which is far away from acceptor emission spectrum and thus acceptor excitation can be minimized (Dong et al., 2012).

In this study, a novel FRET biosensor, with GQD and gold nanoparticle (AuNP) as the energy donor–acceptor pairs, is developed for *S. aureus* gene detection. AuNP is considered to be a kind of highly efficient fluorescence quencher due to its large surface area to volume ratio, easy surface functionalization, and strong surface plasma absorption in NIR-to-IR region (Anger et al., 2006; Zhu et al., 2010). Initially, capture probe and reporter probe was designed based on the sequence specific for *S. aureus mecA* gene and conjugated to GQDs and AuNPs respectively. FRET occurred when both capture probes and reporter probes were exposed to target oligos, allowing the hybridization between complimentary oligonucleotides pairs to bring GQDs to AuNPs into close proximity. Due to the fluorescence energy transfer from GQDs to AuNPs, the quenching of fluorescence intensity was recorded to quantify *S. aureus* target oligos. The detection of limit (LOD) of this GQDs–AuNPs FFET biosensor is around 1 nM for *S. aureus* gene sequence detection. Experiments with single-base-mismatched oligos and double-base-mismatched oligos were also explored and compared with complimentary target oligos to demonstrate the specificity of this FRET biosensor. As a result, this simple FRET biosensor has a low detection limit and good sequence selectivity.

2. Materials and methods

2.1. Materials

Gold (III) chloride trihydrate ($HAuCl_4 . 3H_2O$) and sodium citrate solution were purchased from Sigma Aldrich (St. Louis, MO, US). Graphene quantum dot (GQD) solution was purchased from Nanjing XFNANO Materials Tech Co., Ltd. (Jiangsu, Nanjing, China). 1-Ethyl-3-(3-dimethyl-aminopropyl) carbodiimide (EDC) was purchased from Sigma Aldrich. Oligonucleotides were synthesized and purified by Integrated DNA Technologies (IDT) Inc. (Coralville, IA, US). A 20-base gene sequence fragment of type II Staphylococcal cassette chromosome mecA gene (N315, GenBank: D86934.2) was used as the target (5′-ATTGGGATCATAGCGTCATT-3′). All of these chemicals were used as received without further purification. Thiol-modified capture probe oligonucleotide (A1): 5′-TGATCCCAAT/3ThioMC3-D/-3′, amine-modified reporter probe (G1): 5′-/5AmMC6/AATGACGCTA-3′, 20-mer complementary target probe (C1): 5′-ATTGGGATCATAGCGTCATT-3′, single-base-mismatched target probe (C2): 5′-ATTGGGAT**C**ATAGCGTCATT-3′, double-base-mismatched target to probe (C3): 5′-ATTGGGAT**C**ATAGC**G**TCATT-3′. All oligonucleotides were dissolved in distilled water to prepare stock solution. The bacteria genomic DNA sample was extracted from *S. aureus* strains (ATCC 29213) using an UltraCleanTM Microbial DNA Kit (MoBio Laboratories Inc., Carlsbad, CA). The PCR amplification targeting *mecA* gene was performed in a GeneAmp PCR System 9700 (Applied Biosystems, Foster, USA) with primers of Forward (5′-GATTACTTCAGAACCAGGTCAT-3′) and Reverse (5′-TAAACTGTGTCACACGATCCAT-3′).

2.2. Preparation of gold nanoparticles

Gold nanoparticles (AuNPs) with average size of 15 nm were prepared by the citrate reduction method (Ye et al., 2014). Briefly, a mixture of $HAuCl_4$ (3 μL, 14.3 wt%) and DI water (10 μL) were transferred to a clean beaker which was washed with aqua regia (mixture of HCl and HNO_3 at a 3:1 ratio) and then with DI water. Sodium citrate solution (1 mL, 1 wt%) was added to the boiling solution within one second upon vigorous stirring. The pale yellow solution turned wine red in a few minutes and was boiled for another 15 min. It then cooled down to room temperature by continuous stirring. The final product, AuNPs solution, was suspended in DI water and stored in refrigerator at 4 °C.

2.3. AuNPs–oligo conjugation

The thiol-modified probe oligo (A1: 5′-TGATCCCAA T/3ThioMC3-D/-3′, 64 μM) was treated with DTT (0.1 M, pH 8.2) for 40 min at room temperature to cleave disulfur linkage to enhance efficiency of subsequent oligo immobilization on AuNPs. The activated oligo was purified by gel-columns (illustra Microspin G-25 Columns, GE Healthcare, UK) with centrifugation at 3000 rpm for 2 min and characterized by a UV–visible spectrophotometer (Ultrospec 2100 pro). The purified oligos were mixed with a 500 μL AuNP solution prepared before. The mixture was then incubated at room temperature for 24 h. Then, sodium chloride solution (0.1 M NaCl, 5 mM $NaHPO_4$) was slowly added to allow for 16-h standing. The product was purified by centrifugation at 13,200 rpm for 30 min. The remaining red precipitate at the bottom was collected and rinsed 3 times with DI water to remove the unreacted reagents.

2.4. GQD–oligo conjugation

The purchased GQD solution (1 mg/mL) was firstly sonicated for 10 min. EDC (27 mM) solution was then added into the GQD suspensions. The mixture was shaken on a vortex mixer for 2 min and bath sonicated for another 15 min. After that, amine-modified capture probe (G1: 5′-/5AmMC6/AATGACGCTA-3′, 4.7 μM) was added into the mixture and incubated for 40 min at room temperature. The final product, GQD–oligo conjugate, was characterized by a spectrophotometer equipped with a 450 W steady-state xenon lamp (Edinburgh FLSP920).

2.5. FRET quenching

To investigate the effect of target oligo concentrations on fluorescence quenching efficiency, a fixed amount (1 mg/mL) of GQD-capture probe was incubated with different target oligo concentrations (100 pM to 400 nM) for 2 h to allow hybridization at room temperature. A 50 μL solution of the above mixture was then mixed with a 50 μL reporter probe modified AuNP solution for incubation of 2 h at room temperature to form a co-hybridized sandwich complex structure. After incubation, the fluorescence intensity was measured using a Tecan Infinite F200 micro-plate reader. All the fluorescence intensity was recorded under the same condition. The specificity of the system was also evaluated with both single mismatched oligos and double-base mismatched oligos.

2.6. Characterization

The morphology and size of GQDs and AuNPs were characterized using a JEOL-2100F transmission electron microscopy (TEM) equipped with an Oxford Instrument EDS system, operating at 200 kV. Samples for TEM were prepared on holey carbon coated 400 mesh copper grids. A UV–visible spectrophotometer (Ultrospec 2100 pro) was used for AuNPs adsorption spectra measurement. A FLS920P Edinburgh analytical spectrophotometer was used for GQD emission spectra measurement.

Please cite this article as: Shi, J., et al., Biosensors and Bioelectronics (2014), http://dx.doi.org/10.1016/j.bios.2014.09.059