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A new aptamer/graphene interdigitated gold electrode piezoelectric sensor for rapid and specific detection of *Staphylococcus aureus*

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ABSTRACT

A novel aptamer/graphene interdigitated gold electrode piezoelectric sensor was developed for the rapid and specific detection of *Staphylococcus aureus* (*S. aureus*) by employing *S. aureus* aptamer as a biological recognition element. 4-Mercaptobenzene-diazonium tetrafluoroborate (MBDT) salt was used as a molecular cross-linking agent to chemically bind graphene to interdigital gold electrodes (IDE) that are connected to a series electrode piezoelectric quartz crystal (SPQC). *S. aureus* aptamers were assembly immobilized onto graphene via the π–π stacking of DNA bases. Due to the specific binding between *S. aureus* and aptamer, when *S. aureus* is present, the DNA bases interacted with the aptamer, thereby dropping the aptamer from the surface of the graphene. The electric parameters of the electrode surface was changed and resulted in the change of oscillator frequency of the SPQC. This detection was completed within 60 min. The constructed sensor demonstrated a linear relationship between resonance frequency shifts with bacterial concentrations ranging from $4.1\times10^{1}-4.1\times10^{5}$ cfu/mL with a detection limit of 41 cfu/mL. The developed strategy can detect *S. aureus* rapidly and specifically for clinical diagnosis and food testing.

1. Introduction

Staphylococcus aureus is a gram-positive, widely distributed bacterium, found in air, water and inadequately treated food. *S. aureus* is an important food-borne and iatrogenic pathogen that causes a wide range of diseases, including septicemia, gastrointestinal tract infections, food poisoning toxic shock syndrome and endocarditis under uncontrolled conditions (Rooijakkers et al., 2005).

S. aureus can be detected via many methods. Traditional culture-based assays are time-consume(requiring 2–4 days). Rapid and automated detection methods, including polymerase chain reaction (PCR), enzyme-linked immunosorbent assay, nucleic acid-based molecular biology methods, have been developed (Carroll, 2008; Kipp et al., 2004; Bennett, 2005). They are relatively time-saving (requiring 1–5 h) (Wellinghausen et al., 2009; Jeyaratnam et al., 2008). However, these methods have some shortcomings, such as low automation degree, expensive instruments and complicated sample pre-treatments. Therefore, a novel rapid, specific and sensitive method for the detection of *S. aureus* is needed.

Graphene is a new type of carbon materials with remarkable electrical, thermal and mechanical characters (He et al., 2010) and has been widely used in biosensors (Dreyer et al., 2010; Liu et al., 2013a, 2013b; Sheng et al., 2012; Deng et al., 2012). In particular, graphene, which is a two-dimensional (2D) monolayer of carbon material, has attracted increasing interest owing to their strong $\pi-\pi$ interactions compared to other carbon materials (Moumita and Anil, 2013). Among all kinds of various graphene-based materials, graphene oxide (GO), an aqueous dispersible oxygenated derivative of graphene (Yun et al., 2012), has been widely successfully utilized in molecular hybrids (Yang et al., 2010) or biocompatible (Liu et al., 2010) scaffolds or substrates, and patterned carbon films after being chemically reduced or modified to tune the material properties.

Aptamers are single-stranded oligonucleotides or peptides (typically DNA or RNA) that are selected in vitro using a method known as the systematic evolution of ligands by exponential enrichment (SELEX) (Tuerk and Gold, 1990) from a library of nucleic acids containing $\sim10^{15}$ individual sequences. Aptamers react with their targets (proteins, small molecules, ions, and even cells) with high affinity and specificity, similar to antibodies (Bunka and Stockley, 2006). Aptamers possess additional advantages compared to antibodies: aptamers are easily synthesized, have a higher specificity and stability and a wider range of targets, are inexpensive, and can be simply modified with functional groups. Aptamers can be applied for both therapeutics and diagnoses. A large number of aptamers have been used in fluorescent, (Breaker, 1997; Duan et al., 2012; Wu et al., 2011; Chang et al., 2010),

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colorimetric (Xia et al., 2010), electrochemiluminescence (Hamula et al., 2006) and potentiometric (Zelada-Guillén et al., 2012; Bai et al., 2012) sensors for targets detection.

SPQC technology is highly sensitive, has a low cost and is easy to operate. IDE connected to piezoelectric quartz crystal in series was successfully used to detect *Pseudomonas aeruginosa* in our group (He et al., 2007). Changes in the electrical parameters can be efficiently detected because the steady state can be established quickly in small distances between the anode and cathode electrode systems and because the signal-to-noise ratio was improved (Varshney and Li, 2009; Thomas et al., 2004).

In this study, a novel aptamer/graphene interdigitated gold electrode piezoelectric sensor for *S. aureus* was constructed by immobilizing *S. aureus* aptamers as recognition elements on the graphene surface of IDE. Graphene was immobilized onto the surface of IDE through 4-mercapto-benzenediazonium tetrafluoroborate; which the bonding mechanism of diazonium salt to the graphene surface has been clearly described by Cui et al. (2011). This sensor is rapid, simple, free-labeled, and high sensitive and selective, offering a valuable method for the fast and specific detection of *S. aureus*.

2. Experiments

2.1. Materials

99.95% Graphite powders (325 mesh, Alfa Aesar, USA); 4-Amino Thiophenol (Alfa Aesar, USA). Isoamyl nitrite (Sinopharm Chemical Reagent Co., Ltd., China). Fluoroboric acid (Tianjin Kermel Chemical Reagent Co., Ltd., China). Tris–HCl buffered solution (50 mM pH=7.4, with 5 mM KCl, 100 mM NaCl and 5 mM $MgCl_2$).

All of the regents were pure analytical grade. Deionized water was used for the preparation of all of the solutions.

S. aureus aptamers were collected from the Shanghai Sangon Biological Science and Technology Company Co., Ltd (Shanghai, China). The DNA sequence of the *S. aureus* aptamers was 5′-GCAATGGTACGGTACTTCCTCGGCACGTTCTCAGTAGCGCTCGCTGGTCATCCCACAGCTACGTCAAAAGTGCACGCTACTTTGCTAA-3′.

2.2. Apparatus

^{1}H NMR spectra (Varian INOVA-400 instrument); Raman spectra (BWS 435-785sy Confocal Raman spectrometer, USA); FT-IR spectrophotometer (Thermo Nicolet 5700); SEM (Hitachi S4800); IDE-SPQC detection system (made by ourselves, Fig. S1).

2.3. Stock and test culture

Improved YC medium (yeast extract, 2 g; beef extract, 1 g; D-glucose, 40 g, 18AA amino acids, 16 mL; deionized water, 1000 mL; pH 7.2 ± 0.2; our laboratory).

2.4. Bacterial strains and culture media

The quality control strains: *S. aureus* ATCC 25923 was provided by Hunan Children's Hospital. These strains were inoculated from a blood plate into a liquid medium and were grown for 18 h at 37 °C. Then the colonies on the plates were counted to determine the number of colony-forming units per milliliter (cfu/mL).

The used experimental strains were as follows: *Salmonella Typhimurium* (*S. Typhimurium*) ATCC 50761, *Group A Streptococcus* (*GAS*) ATCC 19615, *Pseudomonas aeruginosa* (*P. aeruginosa*) ATCC 27853, *Enterococcus faecalis* (*E. faecalis*) ATCC 51299, *Klebsiella pneumoniae* (*K. pneumoniae*) ATCC 700603 and *Escherichia coli* (*E. coli*) ATCC 25922, provided by Hunan Children's Hospital. The bacteria were cultured overnight under aerobic conditions at 37 °C in YC media. The concentrations of the six strains were determined by the poured plate counts (PPC) method.

2.5. Modification of interdigital gold electrodes

The interdigital gold electrodes were sonicated for 5 min in acetone and cleaned by isopropanol and ethanol for 5 min, respectively. The electrodes were then rinsed with deionized water for 5 min and then dried under a stream of nitrogen gas. A 70 mM of MBDT (Fig. S3) of solution in acetonitrile was added dropwise to a flask containing the graphene dispersion (Li et al., 2008; Hummers Jr and Offeman, 1958; Kovtyukhova et al., 1999). The mixture was stirred for 7 h at room temperature. Then, the prepared interdigital electrodes were immersed in the mixture solution for 18 h at 38 °C, after which the electrodes were removed, washed thoroughly with acetonitrile and acetone and blown with nitrogen gas to remove the solvent. Next, the aptamers (5 μL) were added dropwise to the modified electrodes, and incubated at 37 °C for 16 h. The modified electrodes were washed twice with buffer solution (50 mM Tris–HCl pH=7.4, 5 mM KCl, 100 mM NaCl, and 5 mM $MgCl_2$) and then with deionized water to remove the free aptamers.

2.6. Detection of S. aureus by IDE-SPQC sensor

Aliquots (1 mL) of different concentrations of *S. aureus* were added to the test tubes, and the modified electrode was placed into the detected solution. The frequency shift curves were recorded automatically by the SPQC software.

3. Results and discussions

3.1. The frequency shift response characteristics of electric parameter properties

Fig. S1A is the apparatus diagram of IDE-SPQC, its equivalent circuit was shown in Fig. S1B, where, 1 is the equivalent electric circuit model of quartz crystal, C_0, L_q, C_q, R_q are static capacitance, motional inductance, motional capacitance and motional resistance, respectively; 2 is the equivalent electric circuit model of electrode modified with layer films, C_f and R_f are membrane capacitance and membrane resistance of modified layer of electrodes, respectively; 3 is the equivalent electric circuit model of solution, C_s and R_s are equivalent capacitance and equivalent resistance of solution, respectively. Because the solution parameters changed little during the detection process, so the Fig. S1B is simplified to Fig. S1C.

The complex impedance Z of this model:

$$Z = R + jX = \frac{R_f}{1+(\omega C_f R_f)^2} - j\left[\frac{\omega C_f R_f^2}{1+(\omega C_f R_f)^2} - \frac{(\omega L_q^{-1}/\omega C_q)}{(1+C_0/C_q)-\omega^2 L_q C_0}\right] \quad (1)$$

where R is the impedance real part, X is the reactive component, $j=\sqrt{-1}$. According to the phase conditions of oscillation theory, when the phase is zero, the phase angle of oscillation is $-\theta$,$-\theta = \tan^{-1}(X/R)$, make$A = \tan\theta$, there are

$$A + \tan(-\theta) = A + (X/R) = 0 \quad (2)$$

$$\frac{AR_f + \omega C_f R_f^2}{1+\left(\omega C_f R_f\right)^2} - \frac{\omega L_q - (1/\omega C_q)}{1+(C_0/C_q)-\omega L_q C_0} = 0 \quad (3)$$