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A sensitive Potentiometric resolved ratiometric Photoelectrochemical aptasensor for Escherichia coli detection fabricated with non-metallic nanomaterials

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ABSTRACT

In this work, a sensitive potentiometric resolved ratiometric photoelectrochemical aptasensor for Escherichia coli (*E. coli*) detection was successfully fabricated with non-metallic nanomaterials. To avoid the use of precious metals or heavy metals, three-dimensional graphene hydrogel-loaded carbon quantum dots (C-dots/3DGH) and graphene-like carbon nitride (g-C_3N_4) with excellent PEC activity and matched potential were prepared. These two materials were modified onto two adjacent areas on the ITO electrode. By applying different bias voltage, the cathodic current generated by C-dots/3DGH and the anodic current generated by g-C_3N_4 can be clearly distinguished and would not interfere with one another. Then *E. coli* aptamer was modified onto the surface of C-dots/3DGH. In the presence of targets, the binding of *E. coli* with aptamer lead to the steric hindrance greatly increased and the cathodic current decreased significantly. Meanwhile, the anodic current generated by g-C_3N_4 was not influenced and it can serve as a stable reference to evaluate the environmental factors. Therefore, the concentration of *E. coli* can be quantified by the ratio of cathodic current to anodic current, which can effectively eliminate these analyte-independent factors and provide a more precise analysis. In addition, this ratiometric PEC biosensor also showed a good sensitivity and a wide linear range (2.9 cfu/mL to 2.9 × 10^6 cfu/mL).

1. Introduction

As a representative foodborne pathogen, Escherichia coli (*E. coli*) is usually infected by consuming unprocessed or contaminated food and can lead to the outbreak of large-scale infectious diseases (Deisingh and Thompson, 2004), which is a great threat to human health. However, traditional foodborne pathogen detection methods, such as microscopic culture (Siegmund et al., 2013) and polymerase chain reaction (Lawal et al., 2015), are costly and need a long processing time, which cannot satisfy the demands from food safety monitoring and clinical diagnosis. Thereby it is very necessary to develop a fast, simple and sensitive method for *E. coli* detection.

Photoelectrochemistry (PEC) phenomenon refers to a process that the photosensitive material absorbed photons to produce electron-hole pairs and then caused the oxidation-reduction reaction at the interface. In recent year, based on the combination of photoelectrochemistry and traditional electrochemical sensing, PEC sensing technology has rapidly attracted the attention of researchers (Li et al., 2016a; Moakhar et al., 2015; Xu et al., 2017; Zhang et al., 2016; Zhao et al., 2017). Compared with traditional optical methods, PEC detector equipment is simple, low price and easy to miniaturize (Golub et al., 2009; Li et al., 2011) because of the use of electrical signal output (Zhao et al., 2014). Besides the advantages of low cost and simple instrumentation, PEC sensing also has a better sensitivity due to the separation of excitation source (optical signal) and detection signal (electrical signal), which provides a lower background signal and the detection limit can be further reduced (Wang et al., 2009). The relevant experiments demonstrated that the detection limit obtained by the PEC method is usually one order of magnitude lower than conventional electrochemical methods when using the same or similar process (Zhao et al., 2015). It has shown a huge advantage in environmental monitoring, food safety, especially in the field of bioanalysis. A variety of PEC biosensors have been developed to detect the corresponding target on account of different biometric elements. The quantitative detection of the photoelectric sensor usually depends on the change in the intensity of the photocurrent caused by the reaction between the target and the optoelectronic active material or the bio-probe (Li et al., 2016b). However, the photocurrent may be interfered by the environmental factors. Ratiometric sensing is an effective tool to improve the detection reliability and have been widely applied in fluorescence detection (Zhang et al., 2011, 2008; Zhu

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et al., 2017) and electrochemical sensors (Hussein et al., 2017; Feng et al., 2016; Wang et al., 2016). Yet, so far, there is few reports about PEC ratiometric sensing because it is more difficult to distinguish photocurrents from different PEC active material. In a recent research, we have successfully developed a novel potentiometric resolved ratiometric PEC aptasensor (Zhang et al., 2017). By applying different bias voltage, the cathodic current and anodic current from two PEC active materials can be generated in order and would not interfere with one another. The quantification of targets was achieved based on the ratiometric between cathodic current and anodic current, which can effectively reduce the external factors interference on the detection results and improve the accuracy of the photoelectric detection process. Meanwhile, this method can be easily operated with present PEC systems and has no additional instrument requirement, providing a simple and common ratiometric PEC detection approach.

Various nonmetallic PEC active nanomaterials, such as carbon dots (Tan et al., 2017; Tiwari et al., 2017) and carbon nitride (She et al., 2016; Zou et al., 2017), have been paid great attention in recent years. Compared with traditional semiconductor materials, nonmetallic nanomaterials are undoubtedly more environmental friendly and can effectively reduce the potential toxicity both to health and environment. In this study, three-dimensional graphene hydrogel-loaded carbon quantum dots (C-dots/3DGH) and graphene-like carbon nitride (g-C_3N_4) with excellent PEC activity and matched potential were prepared to construct a highly sensitive ratiometric PEC biosensor for *E. coli* detection. Besides a better reliability, this proposed biosensor also exhibited a good performance with a wide linear range from 2.9 to 2.9 × 10^6 cfu/mL with an extremely low detection limit of 0.66 cfu/mL (S/N = 3).

2. Experimental

2.1. Reagents and chemicals

Graphite was purchased from Qingdao Tianhe Graphite Co., Ltd (Qingdao, China). Amino-functionalized aptamer was obtained from Sangon Biotech Co., Ltd (Shanghai, China) and the sequence as following:5′-GCA ATG GTA CGG TAC TTC CCC ATG AGT GTT GTG AAA TGT TGG GAC ACT AGG TGG CAT AGA GCC GCA AAA GTG CAC GCT ACT TTG CTA A-3′-NH_2-$(CH_2)_6$. 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), N-Hydroxysuccinimide (NHS), chitosan(CS), glucose and bovine serum albumin (BSA) were provided from Sigma-Aldrich Co. (St. Louis, MO, USA). Furthermore, other reagents and chemicals were obtained from Sinopharm Chemical Reagent Co., Ltd. Phosphate buffer solutions (PBS, 0.1 M) were prepared from NaOH, Na_2HPO_4, and NaH_2PO_4. Graphene oxide (GO) was prepared using modified Hummers method from graphite powders (Gilje et al., 2007). All other chemicals were of analytical grade, and the aqueous solutions were prepared with doubly distilled water.

2.2. Apparatus

The morphologies of the samples were determined using a H800 transmission electron microscope (TEM, Hitachi, Japan). Crystal structure identification was determined using X-ray diffraction (XRD) with a D8 diffractometer (Bruker, Germany) with high-intensity Cu Kα (λ = 1.5406 Å) radiation. Raman spectra were carried out on an RM 2000 microscopic confocal Raman spectrometer. The FT-IR characterizations of the samples were analyzed with Nicolet Nexus 470FT-I FT-IR spectrometer. All the electrochemical and PEC measurements were performed with a CHI 660B electrochemical workstation (Chen Hua Instruments Co., Shanghai, China) and recorded by a conventional three-electrode system where an indium tin oxide (ITO) as a working electrode, a saturated calomel electrode (SCE) as a reference electrode, and a platinum (Pt) wire as a counter electrode. PEC measurement was carried out in 0.1 M phosphate buffer solution (PBS) at critical voltage and a 500 W Xe lamp (Beijing Chang Tuo) was used as the visible light source with 400-nm cut-off filter placed into the path of the Xe lamp to remove the UV irradiation. The synthesis of carbon nitride was accomplished by using a high temperature furnace (Hefei Ke Jing Materials Technology Co., Ltd.) as well as an ultrasonic machine (Kunshan Ultrasonic Instruments Co., Ltd.).

2.3. Preparation of materials

2.3.1. Three-dimensional graphene hydrogel-loaded carbon quantum dots (C-dots/3DGH) were synthesized as following

Firstly, 300 mg glucose was dissolved in 10 mL graphene oxide (2 mg/mL) to form a homogeneous solution. Then, as-prepared solution was transferred into a Teflon-scaled autoclave as well as heated at 180 °C for 12 h and then cooled to room temperature naturally to generate C-dots/3DGH. Finally, the obtained C-dots/3DGH nanocomposites were washed and freeze dried for 48 h.

2.3.2. The g-C_3N_4 was prepared according to the previously published method (Amiri et al., 2016; Amouzadeh Tabrizi et al., 2017) as follows

In short, 2.0 g melamine powder was transferred into high temperature furnace and heated at 520 °C for 4.0 h under argon condition with a ramp rate of about 3 °C/min. The obtained yellow powder was grounded and used for further characterizations (Supplementary materials, the typical SEM image shown in Fig. S1 A, and the XRD patterns as well as FT-IR spectra of g-C_3N_4 were also presented in Fig. S2. These results were demonstrated the successful preparation of graphitic carbonitride.). At last, 5 mg of g-C_3N_4 was dispersed in 1 mL of isopropanol alcohol with ultrasonic agitation for 2 h to achieve a well-dispersed suspension.

2.3.3. Fabrication of the ratiometric PEC Aptasensor

The ITO glass (1 cm × 3 cm) was cleaned with sonication in water and ethanol, respectively. Furthermore, it was dried under an infrared light. Then, the two previously prepared materials C-dots/3DGH and g-C_3N_4 were modified onto two adjacent areas on the ITO electrode. On one side, about 20 μL of C-dots/3DGH (2 mg/mL) was dropped onto the pretreated ITO and dried in air for an hour. Next, 20 μL of EDC and NHS were respectively added dropwise to the ITO electrode surface (Settu et al., 2017), and, allowed to stand for 50 min to activate the carboxyl group (Han et al., 2015). Then, it was washed by ultrapure water and dried in air. Afterward, 20 μL of 1 μM aptamers were coated on the surface of modified electrode at 4 °C for 12 h and then the unreacted aptamers were washed by buffer solution (pH = 7.4). Finally, 20 μL of 1% BSA was coated on the electrode for an hour to block remaining active sites and then was washed with PBS buffer solution for several times (Li et al., 2017; Qian et al., 2010). On the other side, about 20 μL of g-C_3N_4 (1.5 mg/mL) was dropped onto the pretreated ITO and dried in air for an hour. The obtained ratiometric PEC aptasensor was stored at 4 °C.

2.4. Bacterial strain culturing and plate counting

Bacterial suspension of *E. coli* was cultivated in Luria-Bertani at 37 °C with shaking (250 rpm) for 16 h. Then 1 mL bacterial liquid was taken for the detection of absorbance (wavelength of 600 nm). Next, to study the activity of *E. coli*, 1 mL of the bacterial liquid was removed from the tube for 10-fold successively diluted in PBS solution, and 200 μL of each plated on LB agar plates which were cultivated at 37 °C for 36 h. At last, the number of colony-forming units per milliliter (cfu/mL) could be obtained by counting the colonies of *E.coli* on the plate (Hao et al., 2017a).