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A versatile fluorescent biosensor based on target-responsive graphene oxide hydrogel for antibiotic detection

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ABSTRACT

A fluorescent sensing platform based on graphene oxide (GO) hydrogel was developed through a fast and facile gelation, immersion and fluorescence determination process, in which the adenosine and aptamer worked as the co-crosslinkers to connect the GO sheets and then form the three-dimensional (3D) macrostructures. The as-prepared hydrogel showed high mechanical strength and thermal stability. The optimal hydrogel had a linear response for oxytetracycline (OTC) of 25–1000 μg/L and a limit of quantitation (LOQ) of 25 μg/L. Moreover, together with the high affinity of the aptamer for its target, this assay exhibited excellent sensitivity and selectivity. According to its design principle, the as-designed hydrogel was also tested to possess the generic detection function for other molecules by simply replacing its recognition element, which is expected to lay a foundation to realize the assembly of functionalized hierarchical graphene-based materials for practical applications in analytical field.

1. Introduction

Antibiotics, with the ability to kill or inhibit the growth of bacteria, have gained extensively production and application in human and veterinary medicine worldwide during the past decades (Ur Rehman et al., 2015). However, the booming antibiotics consumption in human and animal medicine can directly or indirectly enter into humans, animals, food, and environment (Gao et al., 2015; Wang et al., 2014a; Zhang et al., 2015), which have been emerging contaminants in the environment with much concern for their possible threats to aquatic environment and human health (Liu and Wong, 2013). Moreover, antibiotic use has increased the frequency of resistance genes (Su et al., 2014), which have been an impending threat to public health all over the world. Tetracyclines are a group of broad-spectrum antibiotics, especially OTC has been extensively used as the veterinary antibiotic to prevent bacterial infections in livestock and increase their growth rate because of its effective antimicrobial properties. Unfortunately, the abuses of tetracyclines have caused accumulation of antibiotics in environmental media and food products (An et al., 2015; Ma et al., 2015) and posed serious implications for human health through food chain transmission (Wang et al., 2015). Consequently, it is urgently needed to provide a simple and effective approach to monitor OTC concentration for early warning. Unfortunately, the previously reported methods, including high performance liquid chromatography (HPLC) (Rabolle and Spliid, 2000), enzyme-linked immunosorbent assays (ELISA) (Aga et al., 2003), Surface Enhanced Raman Scattering (SERS) (Li et al., 2013), and amperometric or antibody-based colorimetric methods (Vega et al., 2007; Weber et al., 2005), suffered from tedious sample pretreatment and analysis procedures, possible false positive signals and low specificity of tetracycline derivatives, thus failing to meet the requirements in the fields of environmental monitoring and food safety. Hence, it is imperative to develop rapid, accurate sensing method equipped with specific recognition elements.

In recent years, aptamers application in analytical fields has received extensive concerns (Liu et al., 2009). In essence, aptamers are a class of small, single-stranded RNA or DNA nucleic acids with unique 3D structures that can recognize and bind to their cognate targets with high specificity and affinity, with dissociation constants typically within the pico- to nanomolar range (Nimjee et al., 2005). Generated by systematic evolution of ligands by exponential enrichment (SELEX), aptamers are often referred to as “chemical antibodies” with the unique features of easy modification, high stability, anti-degradation, and so on (Parekh et al., 2010). Moreover, the outstanding specificity of aptamers, far superior to antibodies, makes themselves attractive in small molecule targets detection (Feng et al., 2014). During recent years, aptamers have been optimized and gradually applied in antibiotic sensors design, including detection of tetracycline (Wang et al., 2014b), OTC (Lu et al., 2015), sulfadimethoxine (SDM) (Liu et al.,

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2014), chloramphenicol (Miao et al., 2016), kanamycin (Guo et al., 2015), and streptomycin (Danesh et al., 2016). Since the aptamer sequences specifically binding to OTC were firstly selected by Niazi et al. (2008), several OTC aptasensors have been reported, including electrochemical, optical and microcantilever methods (Kim et al. 2009, 2010 and Hou et al., 2013). However, these sensors commonly suffered from certain drawbacks, such as unavoidable background colorimetric signal, time-consuming immobilization and washing steps and so on. Therefore, it is still a challenge to develop novel sensing strategy for high-performance determination of antibiotics.

GO hydrogel has been systemically investigated during last several years (Xu et al., 2015), soon after the gelation of GO was first observed during the preparation of large-size GO sheets (Luo et al., 2009). Generally, GO can form a stable aqueous dispersion with a concentration as high as 10 mg/mL (Lin et al., 2011). It is believed that the GO sheets are stabilized in water by edge-bound carboxyl moieties, along with the ample amount of hydrophilic epoxy and hydroxyl groups on their basal planes (Konkena and Vasudevan, 2012). While with the assistance of various promoters, GO sheets can readily self-assemble into hydrogels. Polymers, small ammonium salts, metal ions, aromatic monomer, small biomolecule, as well as sonication and pH and so on were considered to facilitate GO gelation by breaking the electrostatic repulsion balance between the negative charged carboxyl moieties (Li and Shi, 2014). These cross-linkers induce the gelation by different supramolecular interactions, including hydrogen bonding, π-π stacking, static or hydrophobic interactions, and coordination (Bai et al., 2011). Those feasibilities of GO sheets into solid 3D macroscopic hydrogels have been deemed to be conducive to their practical application (Shen et al., 2015), including drug release, water purification, supercapacitors and so on (Li and Shi, 2014). More recently, GO-based macrostructures (hydrogels or aerogels) have also been employed in sensor design. For example, Hoa et al. had designed two glucose sensors based on GO hybrid hydrogel. The hybrids increased both the surface area of 3D networks and the electrocatalytic activity of the redox reactions, which resulted in highly improved glucose sensitivity (Hoa et al., 2016, 2015). Li et al. developed a facile approach to grow Au nanoparticles on highly porous 3D graphene hydrogel with large electroactive surface area and high electrocatalytic activity toward NO oxidation, realizing the in situ detection of NO released from living cells (Li et al., 2015).

Previous sensing applications of 3D macroscopic architectures mainly utilized their electrochemical characteristics. However, the electrochemical measuring techniques require tedious modification of electrodes, which are adverse to their extensive application. Herein, a fluorescent assay based on target-responsive GO-based hydrogel was first time employed for antibiotic detection to the best of our knowledge. The hydrogel can be easily prepared by physically mixing GO sheets, adenosine and aptamers together, avoiding time-consuming immobilization or modification procedures and high temperature and pressure consumption. Moreover, the quantitative detection of OTC was implemented under mild conditions by a facile and fast gelation, soaking, and fluorescence detection process. Based on the design principle, the proposed sensor displayed generic detection function and potential possibility for muti-target detection. Compared with the previously reported methods, the hydrogel sensing platform is believed possessing a certain thermal or chemical stability, which would get a promising application in real environmental samples.

2. Materials and methods

2.1. Materials

The fluorescein amidite (FAM) labeled OTC binding aptamer and SDM aptamer (OTC aptamer: 5′-FAM-CGTACGGAATTCGCTAGCCGAGGCACAGTCGCTGGTGCCTACCTGGTTGCCGTTGTGTGGATCCGAGCTCCACGTG-3′, SDM aptamer: 5′-FAM-GAGGGCAACGAGTGTTTATAGA-3′) were synthesized by Sangon Biotech Co., Ltd. (Shanghai, China) and purified by high-performance liquid chromatography (HPLC). Adenosine, OTC, tetracycline, chlortetracycline, metacycline and doxycycline were purchased from Aladdin Reagent Co., Ltd. (Shanghai, China). SDM was provided by Tokyo Chemical Industry CO., Ltd. Phosphate buffer solution (PBS, 20 mM, pH=7.5) was prepared by mixing the stock solution of Na_2HPO_4 and NaH_2PO_4. All other chemicals were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China) and were of analytical grade without additional purification. All solutions were prepared with ultrapure water (Milli-Q water, 18.2 MΩ · cm) from a Millipore Milli-Q system (Bedford, MA, USA).

2.2. Preparation of GO-based hydrogel

The detailed procedures of preparing typical GO-Adenosine hydrogel are available in Supplementary material.

2.3. Antibiotics detection

All the details for detection processes are recorded in Supplementary material.

2.4. Instruments

In this study, several techniques were used to characterize the hydrogels, and the descriptions of the instruments as well as the conditions can be found in Supplementary material.

3. Results and discussion

3.1. GO-based hydrogelation and detection mechanism

GO hydrogels can be readily prepared by physically mixing GO solution with adenosine. The fast gelation of the GO dispersion in the presence of adenosine may attribute to the strong hydrogen bonding and electrostatic interactions between the adenosine and the GO nanosheets. It can be noted that adenosine contain more than one nitrogen (N)-containing functionality (Fig. S1a in Supplementary material). These nitrogen (N)-containing basic functionalities can accept protons from the carboxylic acid groups (–COOH) of the GO sheets (Fig. S1b in Supplementary material) to participate in acid-base-type electrostatic attraction. Furthermore, nitrogen (N)-containing functionalities of adenosine can form hydrogen bonds with hydroxyl groups (–OH), as well as the carboxylic acid groups (–COOH) of GO sheets. For the detection functional hydrogels, aptamers, as a kind of typical functional single-stranded DNA (ssDNA), were added as recognition element. Single-stranded DNA (ssDNA) has been widely applied in noncovalent functionalization or assembly on two dimensional (2D) GO sheets in previous research (Huang et al., 2015; Zhu et al., 2016). In these GO/DNA composite assemblies, ssDNA chains flatly lay on the surfaces of GO sheets as a result of the strong π-π stacking interactions between the hexagonal cells of graphene and the ring structure of nucleobases in ssDNA (Zhu et al., 2015). The π-π stacking interactions between DNA and GO sheets have been elucidated as an effective driving force for assembling GO sheets