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Electrochemical biosensing based on protein-directed carbon nanospheres embedded with SnO_x and TiO_2 nanocrystals for sensitive detection of tobramycin

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ABSTRACT

A series of nanocomposites comprised of homogeneous mesoporous carbon nanospheres embedded with SnO_x ($x = 0$, 1, or 2) and TiO_2 nanocrystals using bovine serum albumin (BSA) as template followed by calcinated at different temperatures (300, 500, 700, and 900 °C) were prepared, and were denoted as $SnO_x@TiO_2@mC$. Then a novel electrochemical biosensing strategy for detecting tobramycin (TOB) based on the nanocomposites was constructed. The as-prepared $SnO_x@TiO_2@mC$ nanocomposites not only possess high specific surface area and good electrochemical activity but also exhibit strong bioaffinity with the aptamer strands, therefore, they were applied as the scaffold for anchoring TOB-targeted aptamer and further used to sensitively detect trace TOB in aqueous solutions. By comparing the electrochemical biosensing responses toward TOB detection based on the four $SnO_x@TiO_2@mC$ nanocomposites, the biosensing system constructed with $SnO_x@TiO_2@mC_{900}$ (derived at 900 °C) demonstrated the highest determination efficiency, high selectivity, and good stability. In particular, the new proposed aptasensing method based on $SnO_x@TiO_2@mC$ nanocomposite exhibits considerable potential for the quantitative detection of TOB in the biomedical field.

1. Introduction

As an aminoglycoside that consists of two or more amino sugars that are connected to a 2-deoxystreptamine scaffold via glycosidic bonds (McKeating et al., 2016b), tobramycin (TOB) exhibits bactericidal activity against a broad spectrum of bacteria, particularly Pseudomonas aeruginosa (Pfeifer et al., 2015). Even TOB is effective in the disease treatment, the use of this kind of aminoglycoside antibiotic has been reduced over the years because of the occurrence of severe side effects including nephrotoxicity and ototoxicity. To obtain both the highest drug efficiency during the disease treatment and the lowest side effects, therapeutic drug monitoring was often performed on patients undergoing therapy with TOB to ensure correct and suitable dosing. Because the side effects mentioned can be caused from the accumulation of TOB in certain organs, the detection of TOB concentration before the next dose of antibiotics is necessary to ensure that they have been properly metabolized and eliminated by the patient. Therefore, it is vital significance to determine the TOB concentration within the blood stream at two different time intervals during the procedure of treatment. However, detection of TOB reservation in aqueous solutions is complicated due to low detection sensitivity, poor chromophore effects, and poor determination stability (Chen et al., 2014). Therefore, considerable efforts have been focused on developing reliable and sensitive methods for TOB quantification to obtain the optimal therapeutic effect. These methods include high-performance liquid chromatography (HPLC) (Manyanga et al., 2013), radioimmunoassay (Prayle et al., 2016), microbiological assays (Orizondo et al., 2016), electrochemical detection (Schoukroun-Barnes et al., 2014), fluorescence resonance energy transfer assays (McKeating et al., 2016a), and colorimetric assay (Pang and He, 2016). Owing to the lack of chemical unsaturation of the TOB molecule, these methods commonly require a derivatization step (pre- or post-column), thereby leading to several disadvantages, such as complexity, time consumption, and high cost. Thus, sensitive and selective strategies should be constructed to determine the trace amount of TOB in the analysis of clinical and biological samples. Among these techniques,

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electrochemical methods exhibit advantages of fast response, high sensitivity, low cost, miniaturization, and on-site analysis; thus, these methods present a promising alternative for the rapid detection of antibiotics (Manyanga et al., 2013).

Recently, the use of nucleic acid aptamers in the fabrication of chemical and biochemical sensors is a rapidly expanding field. In this approach, aptamers are single-stranded DNA or RNA oligonucleotides, which are selected in vitro by a process known as systematic evolution of ligands by exponential enrichment (Šmuc et al., 2013). Aptamers with strong secondary structures, such as a hairpin, can reassemble in the absence of the ligand, leading to high blank signals. Therefore, single-site binding approaches are the only option when small molecules are assayed. Due to the advantages of high affinity and specificity, good stability, and low cost, aptamers have been widely used for the detection of biomolecules (Kimoto et al., 2013; Zhou et al., 2014). With the development of the SELEX (systematic evolution of ligands by exponential enrichment) and PCR (Polymerase Chain Reaction) techniques, the TOB-targeted aptamer chain was synthesized (Spiga et al., 2015) and was employed to determine the trace of TOB (González-Fernández et al., 2013). Combining the advantages of electrochemical techniques and the aptasensors, the electrochemical aptasensors have been exploited and used in many fields, including food safety testing, environmental monitoring, biological diagnosis, and so on.

To improve the determination efficiency of electrochemical biosensors, one key step is the development of new electrode materials, which should possess high bioaffinity interaction toward the biomolecular probe and electronic conductivities (Zhu et al., 2014). With the development of nanotechnology in the recent decades, nanomaterials have received considerable research attention in electrochemical biosensors, given that these materials can meet the above requirement for electrodes. Elsewhere, the chemical stability of these oxides and the capacity to create surface −OH dangling bonds make them relevant materials for covalent grafting of probe molecules in view of biosensing applications (Chen et al., 2010). Therefore, a large number of reports on the applications of nanomaterials as the sensitive layers for detecting the trace analytes were observed (Kumar et al., 2015). Among numerous nanomaterials, carbon materials garner considerable interest in various electrochemical applications, such as sensing, electronics, energy storage and conversion devices, and biotechnology (Fu et al., 2014). In studies related to these fields, various carbon materials, including graphene, carbon nanotubes, and ordered mesoporous carbon (OMC), have been developed (Hernández-Ibáñez et al., 2016; Kim et al., 2016; Zhou et al., 2016). Accordingly, OMC is widely used for biological analysis, electrode materials, guest molecules, and supercapacitors. However, most unmodified carbon nanomaterials are difficult to steadily fix at the electrode surface and directly immobilize biocomponents (Tran and Mulchandani, 2016). Recently, various approaches have been developed for the chemical modification of carbon surfaces. The most widely used technique to functionalize carbon surfaces involves oxidization with acids or ozone, through which oxygenated functionalities, such as carboxylic acids, esters, and quinones are generated (Biju, 2014). However, this method may cause low bonding densities and damage to the pore structure during oxidative treatment (Kurbanoglu et al., 2017).

Among these semiconductor oxides, TiO_2 and SnO_2, well-known wide direct band gap *n*-type semiconductors, are considered as the most promising functional materials because of their unique physical and chemical performance, high biocompatibility, high stability in the physiological environment, and good electrochemical activity (Millesi et al., 2015). For example, a SnO_2 nanopillar-based DNA sensor was fabricated to perform in label-free impedimetric DNA detection (Le et al., 2015). An enzyme-free ultrasensitive urea sensor based on SnO_2 quantum dot/reduced graphene oxide (RGO) composite was fabricated, and the sensor exhibited ultrahigh sensitivity (1.38 $\mu A\ fM^{-1}$) and a rapid response time (~5 s) (Dutta et al., 2014). A sensitive aptasensor based on a robust nanocomposite of titanium dioxide nanoparticles, multiwalled carbon nanotubes, chitosan, and a novel synthesized Schiff base on the surface of a glassy carbon electrode was developed for thrombin detection (Heydari-Bafrooei et al., 2016). A photocatalysis-induced renewable graphene-FET (G-FET) biosensor, which was synthesized by layer-to-layer assembly of RGO and RGO-encapsulated TiO_2 composites to form a sandwiching RGO@TiO_2 structure on a prefabricated FET sensor surface, was established for protein detection (Zhang et al., 2016). Combining the advantages of TiO_2 and SnO_2, the related nanocomposite was prepared and applied in various fields (Zheng et al., 2017). Sukeun Yoon's group reported anode materials based on N-doped mesoporous carbon-decorated TiO_2 nanofibers for lithium-ion batteries. These materials exhibited high capacity because of numerous mesopores in N-doped carbon and excellent electron transport through mesoporous carbon-decorated TiO_2 nanofibers (Ryu et al., 2013). Jahel Ali also reported anode materials with extremely long cycle life based on ultra-small SnO_2 particles in micro/mesoporous carbon (Jahel et al., 2014). Although the carbon structure was retained, this functionalization method needs two separated synthesis steps, which thereby complicating the process.

Recently, protein-mediated synthesis of inorganic nanomaterials for the sensing interfaces of electrochemical cytosensors is deemed advantageous because of the following: mild synthetic conditions, such as near room temperature, in aqueous solutions, and at neutral pH; complex, controllable sizes, shapes, morphologies, and crystal structures; and multifunctional, biocompatible resulting products (Cao et al., 2015). A cationic bovine serum albumin (BSA) nanoparticle system incorporating bimetallic Au–Ag luminescent nanoclusters (NCs) was developed for the delivery of the therapeutic suicide gene into HeLa cancer cells (Dutta et al., 2016). A biocompatible nanocomposite, including BSA template Cu nanoclusters (CuNCs@BSA) and single-walled carbon nanotubes was synthesized to fabricate a highly sensitive electrochemical biosensor for paraoxon as an organophosphate model (Bagheri et al., 2017).

According to the formation of the homogeneous nanocomposite composed of biomolecules and metal oxides, the mesoporous carbon embedded with metal oxide crystals can be synthesized directly by calcination at high temperature. Motivated by the abovementioned considerations, we intended to adopt BSA as template to prepare a novel kind of porous SnO_x@TiO_2@BSA nanocomposite, following by calcination under different temperatures. During the calcination procedure, BSA molecules were decomposed into the mesoporous carbon-rich nanostructure, of which the SnO_x and TiO_2 nanoparticles were embedded in the interior of the whole substances. This special nanostructure endow the formed SnO_x@TiO_2@mC nanocomposite with high specific surface area, good electrochemical activity, and exhibit strong bioaffinity with the aptamer. Therefore, it can be explored as the sensitive layer for aptamer strands immobilization and further developed as the biosensors, of which the TOB-targeted aptamer was employed as the model. Combining the advantages of the each component, including the high absorbability, good electrochemical activity of mesoporous carbon (Ma et al., 2012), good biocompatibility and electrochemical activity of SnO_x (Wu et al., 2012), and the biocompatibility of TiO_2 (Wang et al., 2014), the as-prepared SnO_x@TiO_2@mC nanocomposite was applied as scaffold material for TOB-targeted aptamer strand immobilization (Scheme 1). The developed electrochemical aptasensor based on SnO_x@TiO_2@mC nanocomposite exhibited high sensitivity, outstanding selectivity, good ability, and acceptable applicability in actual samples.

2. Experimental section

2.1. Preparation of SnO_x@TiO_2@BSA and SnO_x@TiO_2@mC nanocomposites

The materials and reagents are shown in the Supplementary Information (S1†). The SnO_x@TiO_2@BSA nanocomposite was prepared by using BSA as