Biosensors and Bioelectronics 25 (2010) 2189–2193

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

A silk derived carbon fiber mat modified with Au@Pt urchilike nanoparticles: A new platform as electrochemical microbial biosensor

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ARTICLE INFO

Article history:
Received 11 October 2009
Received in revised form 30 January 2010
Accepted 1 February 2010
Available online 17 February 2010

Keywords:
Escherichia coli
Silk
Carbon fiber
Au@Pt nanoparticles
Biosensor
Toxin

ABSTRACT

We present here a facile and efficient route to prepare silk derived carbon mat modified with Au@Pt urchilike nanoparticles (Au@Pt NPs) and develop an *Escherichia coli* (*E. coli*)-based electrochemical sensor using this material. Silk is a natural protein fiber, and it is abundant with kinds of functionalities which are important in the development of the derived material. The S-derived carbon fiber mat have amino, pyridine and carbonyl functional groups, these natural existent functionalities allow the Au@Pt NPs to self-assemble on the carbon fiber surface and provide a biocompatible microenvironment for bacteria. The Au@Pt NPs modified S-derived carbon fiber is sensitive to detect the *E. coli* activities with a low detection limit, where glucose is used as a preliminary substrate to evaluate them. The performance of Au@Pt/carbon fiber mat based biosensor is much better than that of commercial carbon paper based biosensor. The high sensitivity of this biosensor stems from the unique electrocatalytic properties of Au@Pt urchilike NPs and quinone groups presented in S-derived carbon fiber. This biosensor is also tested for detection of organophosphate pesticides, fenamiphos. The relative inhibition of *E. coli* activity is linear with −log[fenamiphos] at the concentration range from 0.5 mg/L to 36.6 mg/L with lowest observable effect concentration (LOEC) of 0.09 mg/L. The Au@Pt NPs modified S-derived carbon fiber mat possesses high conductivity, biocompatibility and high electrocatalytic activity and be can used as advanced electrode materials for microbial biosensor improvement. The microbial biosensor based on this material shows potential applications in environmental monitoring.

1. Introduction

The rapid and sensitive determination of toxin is extremely important for early, effective environmental management and water quality preservation, especially for those compounds causes serious health threat to human and animals. In addition, some toxic compounds can be considered possible biological warfare agents and used to kill or injure people. Many toxicity analysis methods, including HPLC, GC–MS and biomonitoring methods, have been widely reported (Compton, 1988; Lechelt et al., 2006; Farré and Barceló, 2003). Many of the reported technologies often fail in routine clinical settings because they still require extensive specimen purification, use complex measurement setups, or are not easily scalable for clinical demands. Therefore, fast and selective analytical techniques that more rapidly and effectively determine toxicity contamination or infection are required.

The interest in biosensor for assessing toxicity has emerged the past few years as the most promising alternative for direct monitoring of toxin (Sotiropoulou et al., 2005; Wang et al., 2009a,b; Liu et al., 2009). The amplitude of the biosensor signal (current) is proportional to the level of metabolic activity of the biocatalyst (enzyme, microbe and organism). The toxic substrate will influence the activity of the biocatalyst, so we can detect the toxicity according to the biosensor signal change. A preferred indirect electrochemical biosensing route based on the inhibition of bacteria has been developed by different groups. Various biochemical oxygen demand (BOD) sensors have been modified for indirect online toxicity determination that is generally based on respirographic methods (Rabaey et al., 2003; Cockerham and Shane, 1994). These systems are able to detect the toxin, however, the main drawback associates with the microbial biosensor is the slow electron transfer between the microbial cell wall and the electrode surface. In order to facilitate the electron transfer rate, great efforts have been devoted to effectively overcome the kinetic barriers. Various mediators are used to shuttle the electrons between the microbe and electrode, which called mediated electron transfer (MET). The majority of microbial sensors utilize MET-type bioelectrocatalysis, since it can be applied to most microbes. However the mediator can be harmful to the cell at the high concentration (Liu et al., 2009) and easily physical absorption on electrode surface. So the direct electron transfer (DET) of microbe towards electrode is quite attractive in electrochemical biosensor, which is sensitive to the

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doi:10.1016/j.bios.2010.02.005

changes in the metabolic status of the cellular biocatalyst and simplifies the construction procedure. To our knowledge, there is only one report on the microbial sensor with the DET bioelectrocatalysis microbe. Kim et al. reported a biomonitoring system using microbial fuel cells for detecting the inflow of toxic substances, which applied the electroactive bacteria (EAB) as the biocatalyst. The EAB are able to use the carbon electrode as an electron acceptor (Kim et al., 2007). An important point to improve the microbial biosensor is to increase the sensitivity towards the toxin. This can be realized by using the suitable bacterial strains which are sensitive to toxin. Recently, the direct electrocatalysis towards glucose with *E. coli* as a biocatalyst after a MFC-evolved process has been reported by Zhang et al. (2006, 2007). This MFC-evolved *E. coli* was chosen as the biocatalysts due to its high electroactivity in our study. Another way to improve the performance of electrochemical biosensor is to use the optimized electrode material with high surface area and good electrocatalytical property. The ideal biosensor platform should provide a favorable microenvironment to maintain the microbe activity and minimize barriers of substrate and product. To date, carbon materials have been the good choice for the microbial biosensor construction due to their good biocompatibility in microbial mixture and high specific area. Nevertheless, they have poor conductivity for the electron transfer between microbes and the electrode surface. Some highly conductive metallic materials, such as Au and Cu usually result in denaturalization of redox-active proteins, such as cytochromes. To provide an electrode material with excellent conductivity and biocompatibility for the microbial biosensor fabrication is the objective in this work. The surface chemical modification is the common strategy to improve the electrode surface property (Chen et al., 2008; Sivakumar et al., 2009; Qiao et al., 2008a). Recent advances in nanotechnology have enabled the development of new platforms (Wang et al., 2009a,b) aimed at more sensitive and faster toxic substrate detection. Recently, our group has developed a simple strategy to rapidly prepare Au@Pt urchilike nanoparticles (NPs) with high catalytic activity and high electroactive area, which are favorable to be used as the functional building blocks to assemble the three dimensional electrode (Guo et al., 2008).

Here we report a new, simple microbial biosensor platform that can detect toxic substrate. We obtained carbon fiber mat from the silk fiber. Silk is a natural protein fiber, which contain kinds of functionalities, e.g. carbonyl groups and amino groups. Those functionalities allow the S-derived carbon fiber several advantages over the usual carbon material. The existence of the amino groups on this S-derived carbon fiber mat can act as an electrostatic anchor for absorption of negatively charged Au@Pt hybrid nanomaterials. So we prepared the Au@Pt NPs modified S-derived carbon fiber mat by electrostatic self-assemble technique. To the best of our knowledge, exploring the 3D hybrid nanoparticles supported on C materials for constructing inhibitor microbe-biosensor has not been reported yet.

2. Experimental

2.1. The preparation of Au@Pt NPs modified S-derived carbon fiber electrode

The silk mat was cut from the commercial cocoon, which was brought from the Qiandaohu, Zhangjiang, China. The silk mat was calcinated in N_2 at 800 °C for 1 h (with 2 °C/min), then S-derived carbon fiber mat was obtained. This mat was allowed to cool down to room temperature before it was exposed to air. The Au@Pt NPs was synthesized via the same procedure as that of our previous work (Guo et al., 2008). A two-step process was employed to synthesize quasi-monodisperse Au@Pt urchilike NPs. 0.5 mL of 1 wt% $HAuCl_4$ solution was added to 50 mL of aqueous solution and the solution was heated to boiling with stirring. Then 0.75 mL of 1 wt% sodium citrate was quickly introduced to the above solution. After heating for several minutes, the solution turned to red, indicating the formation of gold NPs. 1 mL of 0.1 M ascorbic acid (excess) was subsequently added to the gold NPs boiling solution, followed by adding 1.25 mL of 1 wt% H_2PtCl_6. After 20 min of heating, the Au@Pt urchilike NPs were obtained. The carbon fiber mat was immersed in the Au@Pt urchilike NPs solution for overnight. The as-prepared Au@Pt urchilike NPs modified S-derived carbon mat was dried at 80 °C to remove the water. Transmission electron microscopy (TEM) measurements were made on a HITACHI H-8100 EM with an accelerating voltage of 200 kV. Scanning electron microscopy (SEM) images were determined with a Philips XL-30 ESEM. The accelerating voltage was 20 kV.

2.2. Bacteria and fuel cell evolved process

Laboratory strain of *E. coli* DH 5α was used in this study. Details on the bacteria growth and activation can be found in previous study (Zhang et al., 2006). Briefly, the original *E. coli* strain was cultivated aerobically at 37 °C on a rotary shaker for 16 h. A single-chamber MFC with a Pt-catalyzed air cathode was used for bacteria activation and electrocatalytic measurements. A carbon paper (without water proofing, E-Tek) electrode was used as the anode. Bacterial culture was pumped through the anode chamber. The fuel cell was discharged under constant-loading mode by connecting the anode and cathode with a resistor (2 kΩ). After the output current decreased to 5% of its original value, the bacterial culture was pumped out. 10% of culture was added into the fresh medium, and then cultivated at 37 °C on a rotary shaker for 16 h. Three such fuel cell discharge and inoculation cycles can produce electroactive *E. coli* strain.

2.3. The preparation of the MFC-evolved E. coli and Au@Pt NPs modified S-derived carbon fiber mat

The Au@Pt NPs modified S-derived carbon fiber mat and Toray carbon paper (without wet proofing; E-Tek) were used as the working electrode. The working electrode poised at a potential of +300 mV vs. Ag/AgCl reference electrode in MFC-evolved *E. coli* culture for 30 min. Experiments were conducted in a constant temperature room (30 °C) in duplicate. Coiled platinum wire and Ag/AgCl (saturated KCl) electrode were used as the counter electrode and the reference electrode, respectively. The electrochemical measurements were performed with an EG&G 273A electrochemical system (Princeton Applied Research, USA). The solution was purged with N_2 for 25 min before electrochemical measurements. Electrochemical impedance spectra measurements were performed over a frequency range of 1 Hz to 100 kHz with a perturbation signal of 10 mV. A series of dilutions of the fenamiphos stock solution was prepared with 50 mM PBS buffer. The MFC-evolved *E. coli* and Au@Pt NPs modified S-derived carbon fiber mat was incubated in fenamiphos for different time periods, for example, 5, 10, 30, 60, or 120 min, then the electrochemical analysis were recorded. The lowest observable effect concentration (LOEC) was also determined. The LOEC was obtained as follows: $LOEC = 3S/m$, where S and m were the standard error in the intercept and the slope of the linear response–concentration plot, respectively.

2.4. The confocal laser scanning microscopy

Biofilm on Au@Pt NPs modified S-derived carbon fiber mat were examined with confocal laser scanning microscopy (Leica Microsystems Heidelberg GmbH, Mannheim, Germany), as previously described (Richter et al., 2008). FITC was chosen to stain