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Sensors and Bioselective Reagents

Integration of a highly ordered gold nanowires array with glucose oxidase for ultra-sensitive glucose detection

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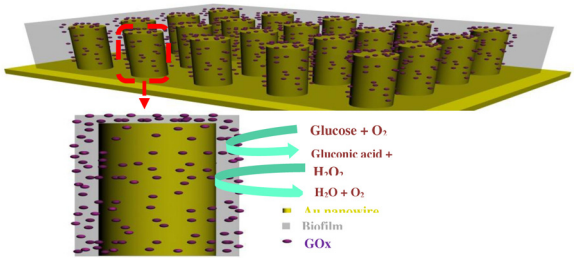
HIGHLIGHTS

- Successfully synthesised highly-ordered gold nanowires array with an AAO template.
- Fabricated an ultra-sensitive glucose nanobiosensor with the gold nanowires array.
- Achieved sensitivity as high as 379.0 μA cm^{-2} mM^{-1} and detection limit as low as 50 nM.
- Achieved excellent anti-interference with aid of Nafion membrane towards UA and AA.
- Enabled successful detection and quantification of glucose in human blood serum.

GRAPHICAL ABSTRACT



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ABSTRACT

A highly sensitive amperometric nanobiosensor has been developed by integration of glucose oxidase (GO_x) with a gold nanowires array (AuNWA) by cross-linking with a mixture of glutaraldehyde (GLA) and bovine serum albumin (BSA). An initial investigation of the morphology of the synthesized AuNWA by field emission scanning electron microscopy (FESEM) and field emission transmission electron microscopy (FETEM) revealed that the nanowires array was highly ordered with rough surface, and the electrochemical features of the AuNWA with/without modification were also investigated. The integrated AuNWA–BSA–GLA–GO_x nanobiosensor with Nafion membrane gave a very high sensitivity of 298.2 μA cm^{-2} mM^{-1} for amperometric detection of glucose, while also achieving a low detection limit of 0.1 μM, and a wide linear range of 5–6000 μM. Furthermore, the nanobiosensor exhibited excellent anti-interference ability towards uric acid (UA) and ascorbic acid (AA) with the aid of Nafion membrane, and the results obtained for the analysis of human blood serum indicated that the device is capable of glucose detection in real samples.

1. Introduction

The considerable development of nanomaterials in recent years has provided several novel platforms for constructing unique electrochemical biosensors that are capable of achieving large active surface area due to their small sizes. Some of the recent applications of nanomaterials in this area include the use of various nanoparticles (gold, platinum, zinc oxide, etc), nanotubes (carbon, titanium

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dioxide, etc), nanowires and nanorods. However, the majority of the efforts on fabrication of biosensors with nanomaterials have mainly focused on the use of nanoparticles and dispersed one-dimensional (1D) nanomaterials. For example, Yang et al. [1] fabricated a glucose biosensor by incorporating Fe_3O_4 nanoparticles to chitosan film and achieved good performance for glucose detection due to the acceleration of the electron transfer by the Fe_3O_4 nanoparticles. Also, TiO_2 nanotubes co-decorated with carbon nanotubes (CNTs) and Pt nanoparticles were successfully utilized to construct amperometric glucose biosensors with improved performance by Pang and collaborators [2]. Besides these examples, various nanoparticles and one-dimensional nanomaterials have been employed to fabricate glucose biosensors [3–8]. In general, glucose biosensors based on one-dimensional nanomaterials, especially ordered one-dimensional nanomaterials, are known to give better performance than those based on the use of nanoparticles [9–15]. However, a noted limitation in this regard is that most of the glucose biosensors based on the use of one-dimensional nanomaterials have focused largely on different kinds of materials such as ZnO nanowires/nanorods array [16–19], TiO_2 nanotubes array [2,20] and noble metal nanowires array [21–23], while largely ignoring other factors such as the morphology and size of 1D nanomaterials array affecting the performance of the glucose biosensors. Also, various approaches such as physical adsorption [16,22] and cross-linking [23] have been utilized for constructing 1D-based glucose biosensors and achieved good results. However, 1D nanostructures array has rarely been explored and utilized in fabricating glucose biosensors. It is worth noting that 1D nanomaterials array also plays a significant role in the improvement of performance of glucose biosensors. Among various 1D nanomaterials, gold nanowires have many advantages, such as large specific surface area, chemical inertness, biocompatibility, excellent electrical conductivity and good electrochemical activity towards H_2O_2 [24–26], which makes this nanomaterial an ideal platform for fabricating nanobiosensors with high performance. Furthermore, it is now well recognized that gold is one the most stable metal at the nanoscale level and this factor may be critical for fabricating highly stable nanobiosensors [27].

In this paper, highly-ordered gold nanowires array with uniform size and morphology were fabricated via well-aligned anodic aluminium oxide (AAO) template (as shown in Supplementary Fig. 1) according to previously reported method [28]. Gold nanowires array with diameter of 80 nm was selected to fabricate glucose biosensors by taking the space between nanowires and its mechanical property into account. In addition, we demonstrated the significant benefits of using well-ordered gold nanowires array (AuNWA) to fabricate a highly sensitive amperometric nanobiosensor for glucose detection by integration with GO_x via cross-linking with a mixture of glutaraldehyde (GLA) and bovine serum albumin (BSA). The ability of the AuNWA to enable improved electron transfer was investigated by cyclic voltammetry with a $Fe(CN)_6^{4-/3-}$ redox system. Also, the morphology and feature of the AuNWA was investigated by field emission scanning electron microscopy (FESEM) and field emission transmission electron microscopy (FETEM). Furthermore, the performance of the resulting AuNWA–BSA–GLA–GO_x biosensor was studied by amperometric detection of glucose and the effect of common interferants, such as uric and ascorbic acids on the performance of AuNWA–BSA–GLA–GO_x biosensor and real sample analysis were also assessed.

2. Experimental

2.1. Materials and reagents

Glucose oxidase (EC 1.1.3.4 181300 units/g from Aspergillus niger) was purchased from sigma (Australia) and used as received. Gold chloride trihydrate ($HAuCl_4{\cdot}3H_2O$), Ethylenediaminetetraacetic acid (EDTA), glutaraldehyde (GLA), bovine serum albumin (BSA), D-(+)-glucose, Nafion (5% w/v) and other chemicals were also obtained from Sigma (Australia). All chemicals were of analytical grade and used as received unless stated otherwise. Milli-Q water (18.2 MΩ cm) was used to prepare all solutions throughout the experiments. Stock solutions of GLA 25% v/v and BSA 20% w/v were prepared and stored in the refrigerator at 4 °C when not in use. Human blood serum was obtained from Jackson ImmunoResearch Laboratories, Inc. (USA) and used for the detection and recovery of glucose.

2.2. Fabrication of gold nanowires array and characterization

The gold nanowires array was grown, according to previously reported approaches [23,29], from a solution which contained 10 g L^{-1} $HAuCl_4$, 5 g L^{-1} EDTA, 20 g L^{-1} K_2HPO_4 and 160 g L^{-1} Na_2SO_3, on the surface of conventional gold electrode directly with the aid of AAO template, shown in Supplementary Fig. 1. The pore diameter and the thickness of AAO template were 80 nm and 16 μm, respectively. Unless otherwise stated, the electrodeposition was carried out with an applied current density of 1 mA cm^{-2}. Prior to electrodeposition, a thin gold film was sputtered onto one side of AAO template by vacuum sputter coater to act as working electrode, and then AAO template was fixed onto gold disk electrode via a Teflon cap, similar to the approach used by Wang et al. [23]. After electrodeposition, AAO template was etched away by utilizing 3 M NaOH to expose gold nanowires array, and then the array was rinsed thoroughly with Milli-Q water and ethanol to remove the residual NaOH. All of the electrochemical depositions were carried out on a galvanostat-potentiostat with a three electrode system at room temperature. Morphology and microstructure of AuNWA was characterized by field emission scanning electron microscopy (FESEM, Hitachi S 4800) and field emission transmission electron microscopy (FETEM, JEOL JEM-2100), respectively.

2.2.1. Immobilization of glucose oxidase and measurement

Glucose oxidase (GO_x) was immobilized onto the surface of AuNWA by cross-linking immobilization with glutaraldehyde (GLA) and bovine serum albumin (BSA). Effect of GLA, BSA and GO_x concentrations on the performance of glucose biosensors based on gold nanowires array were optimized, respectively (As shown in Supplementary Fig. 2(a–c)). Furthermore, other parameters such as drying time, deposition time, buffer solution concentration and pH were also investigated (Supplementary Fig. 3(a–d)). Finally, a 2 μL aliquot of the mixture, which contained 5.5% v/v GLA, 4.8% w/v BSA, 200 U mL^{-1} GO_x, was placed onto the surface of the AuNWA with an area of 0.0095 cm^{-2} and dried for 30 min at room temperature. To enable the assessment of the benefit of the AuNWA, glucose biosensors based on the use of conventional Au disc electrode were also fabricated and tested for comparison. Also to minimise the effect of interferants such as UA and AA, a Nafion layer was formed over the glucose nanobiosensor by placing a drop of 1% Nafion solution on top and left to dry. Amperometric responses were obtained with a galvanostat-potentiostat and cyclic voltammograms (CV) were recorded with a Voltalab PGZ 301 electrochemical workstation. The enzyme electrode, Ag/AgCl (3 M KCl) and Pt/Ti wire were used as working, reference and counter electrodes, respectively.

3. Results and discussion

3.1. Synthesis and morphology of AuNWA

Gold nanowires array (AuNWA) was synthesized with the aid of an anodic aluminium oxide (AAO) template in combination with direct electrodeposition. The diameter of the AAO templates was