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Nanobiosensors prepared by electrodeposition of glucose oxidase in PMMA nanochannels produced by atomic force microscopy nanolithography

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A B S T R A C T

Atomic force microscopy (AFM) nanolithography was employed to fabricate poly(methyl methacrylate) (PMMA) nanochannels with a width of 80 nm, a depth of 30 nm, and a length of up to 8 μm on a Pt-coated indium tin oxide substrate. Quasi-sigmoidal steady-state responses was observed in cyclic voltammograms for the $Fe(CN)_6^{4-}/Fe(CN)_6^{3-}$ redox system in aqueous 0.1 M KCl at the patterned PMMA-coated Pt surface. The PMMA nanochannels were used to prepare glucose nanobiosensors by electrodeposition of glucose oxidase in the presence of Triton X-100. The height of the resulting glucose nanobiosensor was about 80 nm. The prepared glucose nanobiosensors displayed a sensitivity of 6.56 A M^{-1} cm^{-2} and a response time of less than 3 s. The sensitivity obtained in this study is much higher than those obtained at normal macroelectrodes. The present methodology demonstrates the feasibility of electrodeposition of glucose oxidase in PMMA nanochannels produced by AFM nanolithography for biosensing of glucose.

1. Introduction

The miniaturization of electrode has received much attention in the field of electroanalysis [1–7]. Miniaturized sensing devices have several attractive features, including high current density, fast response time, and improved signal-to-noise ratio [8]. Scanning probe lithography (SPL) is used extensively as a nanolithographic tool to fabricate patterns and structures in nanometer scale on material surfaces. It is used as a sharp tip in the end of cantilever to pattern the feature at nanometer scale directly. Among the SPL techniques, mechanical nanolithography is adopted to fabricate nanopatterns for a long time as result of its easy to operate and apply to a variety of substrates such as polymers [9,10]. Here we propose a top–down coupled with bottom–up approach, the combination of atomic force microscopy (AFM) mechanical nanolithography and electrodeposition of glucose oxidase (GOx), to fabricate nanometer scale glucose biosensors on a Pt-coated substrate. The poly(methyl methacrylate) (PMMA) nanochannels were fabricated by AFM mechanical nanolithography, and glucose nanobiosensors were selectively grown in patterned PMMA nanochannels which act as templates by electrodeposition. Pt is a well-known catalyst that has a high catalytic activity for hydrogen peroxide electrooxidation [11]. The growth rate and the dimension of prepared nanostructured materials can be easily controlled by electrodeposition.

2. Experimental

2.1. Reagents

PMMA with a molecular weight of 996,000 g mol^{-1} was purchased from Aldrich. GOx (179 U mg^{-1}) was purchased from Sigma. Triton X-100 was obtained from Acros. Toluene were obtained in the purest commercially available grade and used without further purification. All solutions were prepared with deionized water of resistivity of not less than 18 MΩ cm which was taken from a Milli-Q water purification system (Milli-Q, USA).

2.2. Apparatus

AFM images and mechanical nanolithography were carried out using a SPA-400 (Seiko, Japan) with SPI-3800N in contact mode in air. High-aspect-ratio silicon tips (PPP-RT-NCHR-50, Nanosensors) with a radius of less than 10 nm for the curvature of the tip and a spring constant of approximately 40 N m^{-1} were employed for AFM mechanical nanolithography and imaging. All electrochemical experiments were performed with an Autolab PGSTAT30 Electrochemical Analyzer (Eco Chemie, Netherlands). A conventional three-electrode system was carried out with a prepared nanobiosensor as the working electrode, an Ag/AgCl (3 M KCl) as the reference electrode (Metrohm., Switzerland), and a platinum wire as the counter electrode (Metrohm., Switzerland).

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2.3. Preparation of the glucose nanobiosensors

The Pt film was deposited on indium tin oxide substrate upon a sputtered titanium adhesive layer. A solution of 1 wt% PMMA in toluene was spin-coated (4000 rpm) on a sheet of Pt-coated substrate. After a procedure of 30 min soft baking at 150 °C, the pinholes free PMMA film was prepared. The film thickness of PMMA coated on the Pt-coated substrate was 30 nm which was measured by AFM. An indentation force with vector scan function of AFM nanolithography in contact mode was applied on PMMA film, and then desired nanochannels were created by AFM mechanical nanolithography. The patterned sheet was served as the working electrode for the preparation of GOx nanobiosensors. The preparation of glucose nanobiosensor was performed in 0.05 M phosphate buffer solution (pH 7) containing 20 mg ml^{-1} GOx and 8 mM Triton X-100 at a constant potential of 1.3 V vs. Ag/AgCl for 30 min [12].

3. Results and discussion

The schematic drawing of the general concept for fabrication glucose nanobiosensors is shown in Fig. 1. The PMMA film was prepared by spinning a solution of 1 wt% PMMA in toluene. Next, the nanolithography was performed using AFM in contact mode at a constant speed (0.15 μm s^{-1}). An engraving force of 2100 nN was then decided from the Sneddon formulism of indentation on the basis of PMMA film thickness [13]. Finally, the electrodeposition of GOx was performed in the PMMA nanochannels. To investigate the electroactive behavior at the patterned PMMA surface, cyclic voltammetry was performed. The cyclic voltammograms obtained in aqueous 0.1 M KCl containing 5 mM Fe(CN)$_6^{4-}$ at patterned PMMA-coated Pt surface are shown in Fig. 2. For comparison, a control experiment without the PMMA nanochannels was also carried out. No redox activity is observed for Fe(CN)$_6^{4-}$ at PMMA-
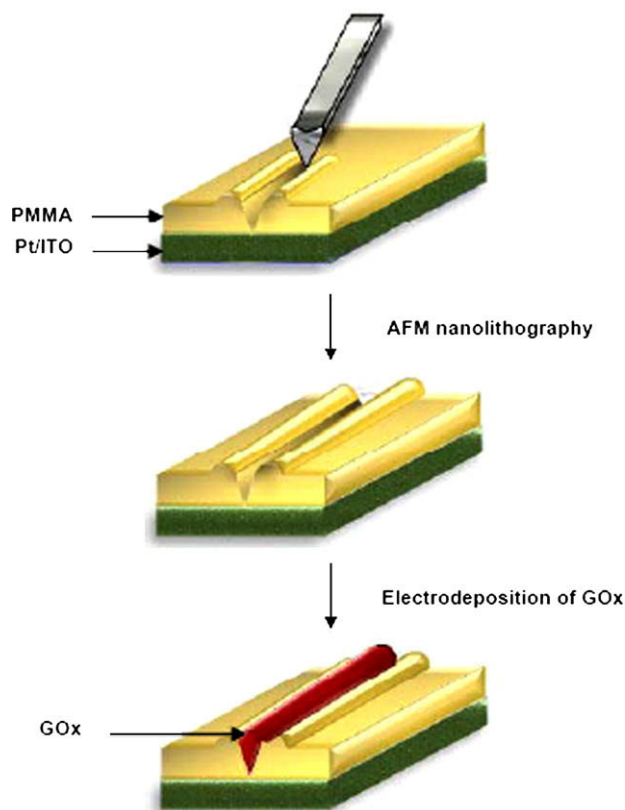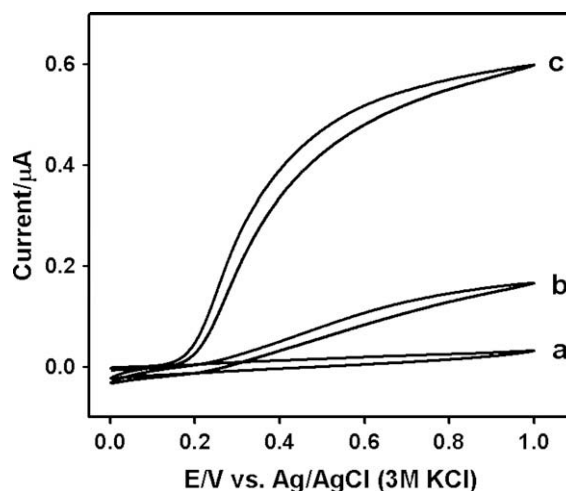

Fig. 2. Cyclic voltammograms of 0.1 M KCl containing 50 mM Fe(CN)$_6^{4-}$ at (a) PMMA-coated Pt substrate and patterned PMMA-coated Pt substrates with nanochannels of (b) 5 and (c) 25. Scan rate: 20 mV s^{-1}.

coated Pt surface (Fig. 2a). The shape of the cyclic voltammogram at the PMMA-coated substrate can be explained with the PMMA film acting as a barrier against the Fe(CN)$_6^{4-}$ to reach the Pt surface. Quasi-sigmoidal steady-state responses are detected and, as expected, with increasing the number of PMMA nanochannels on Pt surface the limiting current increased (Fig. 2b and c). The sigmoidal-shape of the voltammogram is a sign of nanoelectrode behavior (radial diffusion). It is clear from the comparison of cyclic voltammograms in Fig. 2 that higher current response was achieved at patterned PMMA nanochannel with 25 lines. This can be attributed to the larger electroactive access area at patterned PMMA nanochannel with 25 lines. The result demonstrates that it is possible to fabricate nanochannels on PMMA-coated substrate by AFM mechanical nanolithography and expose the underlying Pt layer.

The enzymatic reaction in the use of GOx as a receptor for determination of glucose can be described as follows:

$$Glucose + O_2 \xrightarrow{GOx} Gluconic\ acid + H_2O_2 \qquad (1)$$

$$H_2O_2 \xrightarrow{E} 2H^+ + O_2 + 2e^- \qquad (2)$$
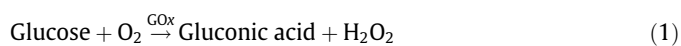
The glucose is catalyzed by GOx in the presence of oxygen and hydrogen peroxide is produced at the same time. The electrooxidation current of hydrogen peroxide is detected after applying a suitable potential to the system. To evaluate the electrocatalytic behavior towards the electrochemical oxidation of hydrogen peroxide at the patterned PMMA-coated Pt surface, amperometry was performed. The amperometric responses for increasing hydrogen peroxide in 0.1 M phosphate buffer solution (pH 7.0) at the patterned PMMA-coated Pt surface are shown in Fig. 3. The current responded quickly and sensitively to the addition of hydrogen peroxide and reached a steady state in 3 s. The effect of the number of PMMA nanochannels on the response currents can be seen in Fig. 3. The response currents increased with increasing the number of nanochannels at patterned PMMA. This result shows that the patterned PMMA-coated Pt surface can be applied to biosensor applications based on hydrogen peroxide detection.

The morphologies of the patterned PMMA nanochannels before and after electrodeposition of GOx are shown in Fig. 4a and b, respectively. It can be seen in Fig. 4a that the depth of the PMMA nanochannel was about 30 nm. The soft resist of PMMA was piled into heaps around the nanochannels can also be observed. The AFM mechanical nanolithography can produce a nanostructure on the

Fig. 1. Schematic drawing of the concept of experimental procedure.