Direct-growth of polyaniline nanowires for enzyme-immobilization and glucose detection

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Abstract

Amperometric enzyme biosensor based on the glucose oxidase (GOx) incorporated polyaniline nanowires (PANI-NWs) on carbon cloth (CC) electrode was demonstrated. The simple, direct-growth of PANI-NWs on CC, via electrochemical polymerization, provides free-standing, template-independent, hence almost (interfacial) defects-free nanostructures. The defect-free interfaces, along with the excellently sensitive organic nanostructured-surface, as evident from its significantly large effective surface area (24 times the geometric area) for redox-sensing, allows efficient entrapment/immobilization and sensing of biomolecules, via rapid electron-transfer at NWs-CC. The GOx-immobilized PANI-NWs/CC, even in initial unoptimized stage, exhibited an excellent sensitivity, \(-2.5\ \text{mA mM}^{-1}\ \text{cm}^{-2}\), to glucose, over detection range 0–8 mM, adequate for clinical monitoring of human glucose levels. The report clearly reveals a cost-effective simple system possessing enormous potentiality for biosensors, bioenergy and bioelectronics applications.

1. Introduction

Recent progress in science bears witness to the sharp rise of one-dimensional (1D) nanostructures due to their unique surface-dominated characteristics and inherent direct transport path, suitable for high-performance devices like chemical/biological sensors, energy devices etc. [1–6]. In research and development of these 1D-devices, the key motivations focus on the surface-functionalization of 1D-structures and their interactions with the analytes/catalysts [4–9]. In biosensor-fabrication, the main challenges lie beneath the immobilization of biomolecules and retention of their bioactivity. In this regard, the organic/polymer 1D-structures exhibits significant advantages over inorganic [9–12], since can provide a compatible environment for the biomolecules-entrapment via electrostatic interaction [9–11,13] and act as a mediator for electrochemical-based biosensor [14].

Polyaniline (PANI) has been extensively studied due to its low-cost, ease in synthesis and interesting electrochemical property [10,13,14]. Generally, the preparation of nanostructured PANI involves polymerization of aniline-monomer in acidic solutions, accomplished either by chemical template-based syntheses [15–17] or via electrochemical deposition routes. In template-based approach, nanostructures-morphology is constrained by the template-topography, resulting in substantial interfacial defects and hence reduced electron-transfer rate. In contrast, the direct-growth via electrochemical deposition methods [18–20] provides a simple low-cost mass-production and, most importantly, template-independent growth, leading to the nanostructures-formation with almost strain-free morphology.

In this study, the direct-growth of PANI nanowires (PANI-NWs) on carbon cloth followed by incorporation of glucose oxidase (GOx) is carried out to fabricate the amperometric enzyme electrodes. Carbon cloth (CC) was specifically selected as the support due to its cost-effectiveness, high conductivity, reasonable chemical stability, and 3D structure with high porosity, hence high surface area for surface-functionalization and PANI-growth. In addition, the flexible nature of CC is also preferable for fabrication of flexible-electrode with ease in designing and packaging. Simultaneously, the direct-growth of PANI-NWs can provide the defect-free interfaces with the support CC, which in turn significantly improve the electron-transfer efficiency between PANI-NWs and CC, therefore enhancing the current response, for PANI-NWs-deposited CC (PANI-NWs/CC) electrodes. Moreover, the large specific surface area of PANI-nanostructures can provide the efficient immobilization of GOx and facilitate a highly sensitive glucose-sensor.
2. Experimental details

2.1. Materials

GOx (E.C.1.1.3.4, Type II from Aspergillus niger, 15,000–25,000 units/g solid) (Sigma) was used in this work. Carbon cloth (B-1, Designation A: plain-weave, 0.35 mm thickness; no wet-proofing), is purchased from E-TEK Division (USA). β-D(+) glucose and other reagents were of analytical grade, purchased from Riedel-de Haën (Hannover, Germany).

2.2. Fabrication of GOx/PANI-NWs/CC electrodes

Fig. 1 presents a schematic diagram for the fabrication of GOx/PANI-NWs/CC electrode. Deposition of PANI-NWs onto carbon cloth (geometrical area \(0.15 \text{ cm}^2\)) was performed by electrochemical oxidation of aniline (0.2 M) in HCl (0.5 M), with controlled coating-efficiency and uniformity. At the nucleation stage of PANI (Fig. 1b), the linear potential sweep voltammetry was performed above 0.6 V (vs. Ag/AgCl RE) for aniline-oxidation. Subsequently, NWs-growth was initiated under galvanostatic condition (at constant current, 1 mA) for 10 min at room temperature (Fig. 1c). The preliminary study revealed that, under the above-mentioned galvanostatic condition, a uniform and dense coverage of PANI-NWs on CC could be achieved. Further rise in current or prolonged oxidation time resulted acute detrimental effect on PANI-NWs, via "peeling off" from the CC-surface. The as-grown PANI-NWs were subjected to reduction process (Fig. 1d) in a phosphate buffer solution (PBS) (pH = 7.3), where the potential was swept to 0.0 V and maintained for 15 min, in order to remove the anions and physically-adsorbed residues from NWs-surface. Then PANI-NWs/CC was transferred to GOx (2.5 mg/ml) solution in PBS (Fig. 1e), and initially subjected to oxidation at 0.25 V for 15 min. As an immediate consequence, the \textit{in situ} immobilization of active GOx was induced (Fig. 1e), via electrostatic interaction between the positive-charged (oxidized) NWs-surface and the negative-charged GOx molecules. The final product, GOx/PANI-NWs/CC electrode, was rinsed thoroughly with PBS solution and stored at 4°C when not in use.

2.3. Characterizations

Microstructure and morphology were investigated using field-emission scanning electron microscopy (FESEM, JEOL-6700), and transmission electron microscopy (TEM, JEM-4000EX). FT-IR spectra were recorded by using Nicolet 6700 Fourier-transform infrared spectrometer. Electrochemical measurements were performed using a potentiostat (Autolab/PGSTAT12), in a conventional three-electrodes configuration with GOx/PANI-NWs/CC as working electrode (WE), platinum wire as auxiliary electrode, and Ag/AgCl (3 M KCl) as reference electrode (RE).

3. Results and discussion

3.1. Characteristics of PANI-NWs/CC composite

SEM observations (Fig. 2A) exhibited a dense distribution of randomly oriented nanostructured PANI on the surface of CC. For comparison, the morphology of the original CC is shown in Fig. 2B. The mechanism for the nanowire-formation can be attributed to the induced concentration-gradient between the solution inside and outside the mesopores during polymerization and the subsequent anisotropic growth process. The high-magnification SEM image (Fig. 2C) illustrated the free-standing wire-like structures of PANI-NWs, while TEM image (Fig. 2D) further revealed the amorphous phase and strain-free morphology, with 60–80 nm in diameter and several microns in length. Such nanostructure of PANI can be expected to provide adequately active-surface for subsequent immobilization of enzyme.

FT-IR spectra (Fig. 2E) exhibited the compositional characteristics of pristine and GOx-immobilized PANI-NWs/CC: characteristic IR modes for PANI, around 1587, 1497, and 1299 cm\(^{-1}\), were observed for GOx/PANI-NWs/CC composites (curve b, marked with \(*)\). Peaks around 1590 and 1490 cm\(^{-1}\) are attributed to imino-1,4-phenylene and quinine dimine structures, respectively, which agree with pristine PANI-NWs/CC (curve a). A comparison with pure GOx (curve c) confirms the incorporation of GOx onto GOx/PANI-NWs/CC (curve b): the absorption peaks at 1652 and 1590 cm\(^{-1}\) (shaded area) are attributed to the stretching of C=O bond and the in-plane vibration of N–H bonds of GOx, respectively. The bands around 1433, 1379, and 1100 cm\(^{-1}\) correspond to CH\(_3\), CH\(_2\) and C–O bending vibration of GOx, respectively (shaded area).

3.2. Nerstian behavior of PANI-NWs/CC electrode

Cyclic Voltammetric (CV) responses (Fig. 3A) for redox reaction of potassium ferricyanide \(K_3[Fe(CN)_6]\) serve as benchmark in

![Fig. 1](image_url)
investigating Nerstian behavior of PANI-NWs/CC (solid line) and the unmodified carbon cloth (CC) (dash line) electrodes. Higher anodic peak current \( I_{pa} \) for PANI-NWs/CC (16.0 mA/cm²), compared to CC (2.47 mA/cm²); suggest highly enhanced redox-response after PANI-NWs-growth, and also the involvement of larger reaction surface area and lower electrical resistance. Dependence of \( I_{pa} \) on the square root of scan-rate exhibited an almost linear relationship (inset, Fig. 3A), indicating a typical diffusion-controlled electrochemical behavior. The value of the effective surface area, for PANI-NWs electrode, is evaluated as 3.58 cm², which is 24 times the geometric area, hence confirms the effectiveness of nanostructures having high surface-sensitivity as a potential electrochemical electrode. Such surface-sensitivity in combination with the recognition property of biomolecules, can establish the viability of GOx-immobilized PANI-NWs/CC as an electrochemical biosensor.

3.3. Electrochemical sensing of glucose

To study the viability of GOx/PANI-NWs/CC electrode as a potential glucose-sensor, CV and amperometric measurements were performed. Fig. 3B (inset) illustrated that, without GOx-mediation,
the PANI-NWs/CC electrode shows almost no activity in presence of glucose; while the GOx/PANI-NWs/CC electrode exhibited a well-defined glucose-oxidation peak around 0.6 V, which increases significantly with the increase in glucose-concentration ($C_G$) (Fig. 3B). Such phenomena supports our expectations (Section 3.2) for PANI-NWs/CC as a highly sensitive glucose-sensor.

Fig. 3C displays a typical amperometric response of GOx/PANI-NWs/CC biosensor with the addition of successive aliquots of 1 mM glucose in PBS, under the constant potential at +0.5 V. The biosensor performed a rapid and sensitive response to each injection of glucose: a sharp rise in current followed by a steady-state value ($I_{ss}$) within 10 s, while no response was observed in analogous measurements with GOx-free PANI-NWs/CC electrode. The plot of $I_{ss}$ versus $C_G$ (inset, Fig. 3C) depicted a linear relationship over the range $0 \leq C_G \leq 8$ mM, an adequate range for clinical applications in monitoring of human glucose levels, until $I_{ss}$ saturates at a high $C_G (>16$ mM). Linear least square fit of the data, taking account of the corresponding experimental errors, concluded the sensitivity of 2.5 mA mM$^{-1}$ cm$^{-2}$, with limit of detection below 0.05 mM, determined at a signal-to-noise ratio of 3. The sensor shows excellent stability (about 82%, as estimated from the successive runs over a span of a week) for several repetitions, under the same detection-condition. As expected, PANI-NWs/CC, even in current unoptimized stage, has substantiated as a potential glucose-sensor, comparable to other conductive polymers-based electrodes [21–25]. It exhibits noticeably excellent performance, especially in the stability and sensitivity (almost two orders of superiority), in comparison with other glucose-sensors [21–25]. Better performance would be anticipated from the further optimization of NWs-fabrication and device-design. Since the performance directly related to the surface-sensitivity of PANI-NWs, the control on the NWs-size should improve the sensitivity and detection limit.

4. Conclusions

The study has demonstrated an efficient and simple route to synthesis PANI-NWs directly on the carbon cloth using electrochemical polymerization method, followed by its application in amperometric enzyme biosensing. Because of the large specific surface area, excellent conductivity and high porosity, PANI-NWs/CC easily provides larger effective surface area to immobilize GOx. Moreover, the reasonably defect-free PANI-NWs/CC interfaces allows rapid electron-transfer and hence enhance the glucose-response. Although the concept has been presented within the context of glucose-sensing, it could be readily extended to other biocatalytic systems based on the ample selection of enzymes.

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References