Applied Surface Science 402 (2017) 136–141

Contents lists available at ScienceDirect

Applied Surface Science

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

Full Length Article

Development and surface characterization of a glucose biosensor based on a nanocolumnar ZnO film

A. Rodrigues[a,1], M.V. Castegnaro[a], J. Arguello[b], M.C.M. Alves[b], J. Morais[a,*]

[a] Instituto de Física – UFRGS, P.O. Box 15051, 91501-970 Porto Alegre, RS, Brazil
[b] Instituto de Química – UFRGS, P.O. Box 15003, 91501-970 Porto Alegre, RS, Brazil

ARTICLE INFO

Article history:
Received 11 August 2016
Received in revised form 5 January 2017
Accepted 6 January 2017
Available online 9 January 2017

Keywords:
ZnO
Nanocolumns
Glucose oxidase
X-ray photoelectron spectroscopy
Synchrotron
X-ray diffraction

ABSTRACT

Highly oriented nanostructured ZnO films were grown on the surface of stainless steel plates (ZnO/SS) by chemical bath deposition (CBD). The films consisted of vertically aligned ZnO nanocolumns, ~1 μm long and ~80 nm wide, as observed by SEM (scanning electron microscopy) and FIB (focused ion beam). XRD (X-ray diffraction) confirmed the c-axis preferred orientation of the ZnO columns, which were functionalized with the glucose oxidase (GOx) enzyme into a biosensor of glucose. The electrochemical response studied by CV (cyclic voltammetry) proved that the biosensor was capable of detecting glucose from 1.5 up to 16 mM concentration range. XPS (X-ray photoelectron spectroscopy) analysis, excited with synchrotron radiation, probed the atom specific chemical environment at the electrode's surface and shed some light on the nature of the ZnO-GOx interaction.

1. Experimental methods

The synthesis of ZnO nanomaterials with tunable size and morphology is one of the key issues for the development of new microelectronic devices, sensors, lasers, etc. Nanostructured ZnO films are currently obtained by both physical and chemical methods, and their properties and potential applications have been widely investigated [1,2]. Although there are many physical methods to prepare ZnO films [3–7], the CDB is a low cost option which, once optimized, provides high quality ZnO films [8].

Furthermore, ZnO is biocompatible and its high isoelectric point (IP) of about 9.5, is suitable for the adsorption of proteins with low IPs, such as GOx, that presents an IP ~4.2 at the pH of 7.4. GOx is employed in glucose biosensors, since it catalyzes the oxidation [9,10] of β-D-glucose, leading to products detectable by electrochemical measurements. The immobilization of GOx onto a nanostructured ZnO electrode is primarily driven by the electrostatic interaction between the enzyme and the ZnO surface [9,10].

ZnO films grown on a variety of substrates, such as silicon, tin-doped indium oxide (ITO) and gold foil have been currently employed in the development of glucose sensors [9,11,12]. On the other hand, the use of stainless steel as a substrate for ZnO in a glucose biosensor electrode is not extensively explored. Nevertheless, stainless steel (SS) is an attractive option for that purpose due to its biocompatibility [13] associated with its high corrosion resistance, high conductivity, and thermal stability. Recently, ZnO/SS films were successfully obtained and evaluated as an alternative flexible material for resistive random-access memories (ReRAMs) [14].

XPS is an important surface science tool that enables to probe the chemical environment of selected atoms at the surface of any material. The use of high intensity monochromatized X-rays, provided by synchrotron radiation sources enables to detect chemical shifts associated with specific chemical interactions. Although XPS was already applied in ZnO-based biosensors investigations [11,15,16], to our knowledge there are no comprehensive reports on the use of XPS to elucidate the chemical interaction of the GOx with the ZnO surface.

This paper reports on the development and characterization of a glucose sensor based on a nanostructured ZnO film grown directly on a stainless steel substrate (ZnO/SS) by CBD. SEM and FIB were applied to assess the film morphology, while XRD was used to evaluate its crystalline structure. The glucose biosensor was prepared by immobilizing the glucose oxidase onto the ZnO nanostructures,

* Corresponding author.
E-mail addresses: adriana.rodrigues@partner.kit.edu (A. Rodrigues), jonder@if.ufrgs.br (J. Morais).
[1] Present professional address: Synchrotron Facility ANKA, Karlsruhe Institute of Technology KIT, Hermann-von-Helmholtz-Platz 1, 76344 Eggenstein-Leopoldshafen, Germany.

http://dx.doi.org/10.1016/j.apsusc.2017.01.052
0169-4332/

and CV was applied to test the developed biosensor. Synchrotron radiation excited XPS was used to probe the surface of the ZnO film, before and after the immobilization of the GOx.

2. Experimental methods

2.1. ZnO/SS film deposition

The nanocolumnar ZnO/SS film preparation was based on a previously reported procedure [17] using zinc acetate (Vetec), hexamethylenetetramine (HMT-Aldrich) and diaminopropane (DAP-Aldrich). Deionized water was used as solvent to prepare the reaction solution by mixing the solutions of 20 mM zinc acetate, 20 mM HMT and 100 mM DAP. A stainless steel piece ($3 \times 3\,mm^2$), was cleaned with acetone and isopropyl alcohol. Prior to the ZnO film growth, a 5 mM solution of zinc acetate in ethanol was placed dropwise on the stainless steel surface and dried at room temperature. It was then heated at 200 °C in air for 20 min to form a seed layer over the substrate. The stainless steel substrate was then vertically submerged in the reaction solution inside a autoclavable bottle. The bottle was sealed and immersed in a thermal bath at 95 °C during 8 h. At the end of the reaction, the substrate was thoroughly washed with deionized water to remove chemical residues, and dried at room temperature.

2.2. Immobilization of GOx

A phosphate buffer solution (PBS) was prepared with 0.01 M KH_2PO_4 and $Na_2HPO_4{\cdot}7H_2O$ in deionized water. Then 6 mg of GOx (Sigma-Aldrich) was added to 1 mL of the buffer solution (pH 7). The ZnO/SS electrode was washed with the PBS solution, dried under nitrogen flow, immersed in the GOx solution, and kept in a refrigerator for 24 h at 4 °C. After this period, the electrode was washed with deionized water to remove the excess of GOx.

2.3. Electrochemical measurements

The performance of the glucose biosensor was investigated in PBS solution by the addition of 1.5 mM up to 16 mM of glucose. The experiment was carried out in a three electrodes cell arrangement, using the GOx/ZnO/SS plate as the working electrode, a Pt grid as the counter electrode, and a saturated calomel as the reference electrode. Cyclic voltammetry (CV) measurements were performed using an Autolab PGSTAT 30 potentiostat, in the potential range from 0.2 to 1.0 V at various scan rates: 10, 20, 40, 50, 80 and 100 mV s^{-1}.

2.4. Characterization

The crystalline structure of the ZnO/SS film was analysed by XRD with a Diffraktometer D500 Siemens using Cu Kα radiation (λ = 1.5406 Å, 17.5 mA, 40 kV). The thickness of the ZnO film was evaluated by investigating the image of the crater formed by ion bombardment with a FIB equipment (Jeol model JIB 4500, 30 kV Ga^+ ions). For the TEM and HRTEM, part of the film was carefully removed and well dispersed in 0.5 mL of isopropil alcohol. One drop of the suspension was placed on a holey carbon-coated copper grid and allowed to dry in air. The grid was then observed on a JEOL JEM 2010 microscope working at 200 kV. The morphology of the ZnO/SS film, before and after the GOx immobilization, was observed by SEM using a JEOL JSM 6060 microscope working at 20 kV. The average diameter of the ZnO columns was estimated from ~800 counts chosen in arbitrary areas of the SEM images using the ImageJ 1.45 software. The presence of the enzime on the top of film was investigated by Energy-dispersive spectroscopy (EDS) analysis using a JEOL JSM 5800 microscope.

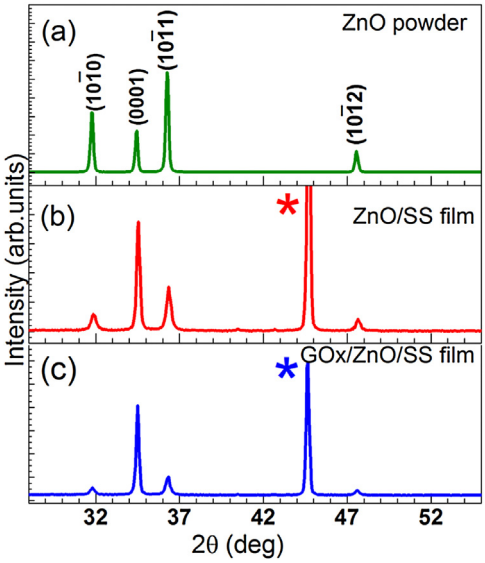

Fig. 1. XRD patterns of the (a) standard ZnO, (b) the ZnO/SS and (c) GOx/ZnO/SS films. The peak at about 45° (*) is a contribution of the substrate.

The XPS analyses were performed using photons with 1840 eV of the SXS beamline at the LNLS (Brazilian Synchrotron Light Laboratory), at room tamperature and 45° takeoff angle. The base pressure inside the analysis chamber was about 8×10^{-10} mbar. The hemispherical analyzer Phoibos 150 SPECS was set at a pass energy of 30 eV and an energy step size of 0.1 eV. For energy calibration, it was used as reference the Au $4f_{7/2}$ photoemission peak (84 eV) [18] measured from a clean standard Au foil. The data analysis procedure using XPSPEAK 4.1 considered a Shirley type background and a Gaussian-Lorentzian sum function (20% Gaussian) for peak fitting. The C 1s binding energy (284.5 eV) [18] was used as an internal reference in order to correct minor charging efects. The ZnO/SS film was analysed before and after ion sputtering with an Ar^+ beam (1.5 kV, normal incidence) for 30 min at a preasure of 1.5×10^{-6} mbar. Photoemission data was also collected after the immobilization of GOx onto the ZnO/SS film.

3. Results and discussion

The XRD results (Fig. 1) of the ZnO/SS and GOx/ZnO/SS films are compared with a reference ZnO powder (Riedel-de Haën), which is indexed to the ZnO wurtzite structure (JCPDS, card N°. 36.1451). The XRD pattern of the ZnO film is dominated by the (0001) reflection due to a highly preferred growth in this direction. No changes were observed after the enzyme immobilization (Fig1(c)).

The FIB image (Fig. 2(a)) displays the crater formed by the ion beam, which reveals the vertically aligned columns with $\sim 1.13 \pm 0.10\,\mu m$ length. The TEM image of the isolated nanocolumns (Fig. 2(b)) shows a wider lower base that narrows down towards its tip. Well-defined lattice fringes are observed in the HRTEM image of the tip region (Fig. 2(c)). The distance between two parallel fringes is ~0.52 nm, which corresponds to the (0001) lattice spacing of the wurtzite hexagonal ZnO. This confirms that the columns are single crystalline and preferentially grown along the (0001) direction. The Fourier transform (FT) pattern (inset in Fig. 2(c)) corroborates the c-axis orientation of the ZnO.

The SEM image of the ZnO/SS film (Fig. 3(a)) shows a dense growth of columns with an average diameter of $\sim 75 \pm 15$ nm. The image of ZnO film after the GOx immobilization is blurred (Fig. 3(b)), due to the presence of the adsorbed enzyme layer over the ZnO nanocolumns. EDS analysis of the plain ZnO surface (inset in Fig. 3(a)) reveals the presence of Zn and O from the film, as well as