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Towards Nanostructured ITO-Based Electrochemical Sensors: Fabrication, Characterization and Functionalization †

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Abstract: The need for miniaturized, low-cost and ultrasensitive electrochemical sensors has motivated the search and study of new nanostructured materials. We propose nanostructured indium tin oxide (ITO) electrodes as a promising platform due to their good electrical conductivity, transparency to visible wavelengths and high surface-to-volume ratio. The nanostructured electrodes were fabricated by electron beam evaporation, and electrochemical techniques were used to quantify more than a 40% increase in electrochemical surface area compared to thin ITO films. The electrodes were derivatized with organosilanes and coated with a molecule providing redox activity. Indeed, an increase in detectability of more than 400% was observed with respect to thin films, indicating the potential viability of nanostructured ITO-based electrochemical biosensors.

Keywords: indium tin oxide; ITO; cyclic voltammetry; organosilane; XPS; FTIR; 6-(ferrocenyl)hexanethiol; electrochemical sensors

1. Introduction

Due to the increasing need for miniaturized, low-cost and ultrasensitive electrochemical sensors, new nanostructured materials are being considered for their high surface-to-volume ratio and enhanced electrocatalytic activity [1]. Besides, the most promising detection techniques for biosensors involve electrochemistry and optical absorption. In this respect, transparent conductive oxides (TCOs) have become a feasible alternative to other structures [2], since they have long been fabricated by standard complementary metal oxide semiconductor (CMOS) processes.

In particular, indium tin oxide (ITO) has recently been used as thin film electrodes in electrochemical biosensors [3] owing to its good electrical conductivity and high transparency to optical wavelengths, resulting in a good candidate for either electrochemical and/or optical biosensors. Few studies on the utilization of thin ITO films as biosensing electrodes can be found in the literature. Taking advantage of the combined optical and electrical properties of ITO, Choi et al. developed a system capable of obtaining optical images while at the same time performing microimpedance analyses of time-dependent cellular growth [4]. This system was useful to correlate impedance increases with higher cell-covered area increases on the electrode. Same authors reported later an improved system to determine the dynamic cellular attachment, spreading and proliferation of endothelial cells [5]. Indium tin oxide has been used recently in combination with gold for the development of an electrochemiluminescent biosensor for detecting cancer cell surface proteins [6].

We present a study on the feasibility of developing nanostructured ITO electrodes for electrochemical biosensors as a viable alternative to CMOS-non-compatible materials (enabling low-cost and high throughput fabrication) and as a way to enhance sensors sensitivity due to surface

nanostructuration. First, the nanostructured surfaces were characterized in terms of electrochemical surface area available for attachment of molecules in comparison with thin ITO films. Second, the substrate derivatization with an alkylsilane molecule was proved successful by surface characterization with X-ray photoelectron spectroscopy (XPS) and Fourier transform infrared spectroscopy (FTIR). At this level, a ferrocene-labeled molecule was attached to the derivatized surface as a proof of concept.

2. Materials and Methods

2.1. Growth of ITO Electrodes

The ITO samples were grown by electron beam evaporation on crystalline N^+-doped silicon substrates with a Pfeiffer Vacuum Classic 500. Commercial ITO targets were purchased from Neyco, France. Substrate temperature was set at 100 °C and 500 °C for thin ITO (tfITO) and nanostructured ITO (nITO) films, respectively. Deposition rate was set at 1 Å/s during a total time of 2000 s. After the evaporation process, the samples were annealed at 600 °C for 1 h in nitrogen atmosphere to promote the ITO crystallization for optimal electrical conductivity and optical transparency. SEM images of the electrodes revealed nanowires with a mean diameter of 30 nm [7,8].

2.2. Electrochemical Characterization

Cyclic voltammetry was used to determine the electrochemical surface area by faradaic current analysis. To do so, subsequent voltammograms at different scan rates were performed in aqueous media, with $Fe(CN)_6^{3-/4-}$ as redox couple at 5 mM and 0.1 M KCl as electrolyte. The electrochemical experiments were performed with a Biologic-EC-Lab SP150 potentiostat.

2.3. Substrates Functionalization

A solution of 3-glycidoxypropyltrimethoxysilane (GPTMS) at 4% (*v*/*v*) in dry toluene was used for ITO derivatization. The reaction was let take place overnight at room temperature and under soft mixing. The GPTMS molecule has an epoxy ring termination that is very reactive to molecules containing thiol-, amine- or hydroxyl-ligands. In the present study, 6-(ferrocenyl)hexanethiol was used as a proof of concept. A solution of the latter was prepared at 500 μM in *N*,*N*-dimethylformamide (DMF).

2.4. X-ray and Infrared Spectroscopies

X-ray photoelectron spectroscopy (XPS) was performed in a PHI Multitechnique System (Physical Electronics) with a monochromatic X-ray source (Al Kα line of 1486.6 eV and 350 W) in ultra high vacuum chamber pressure between 5×10^{-9} torr and 2×10^{-8} torr. Fourier transform infrared spectroscopy (FTIR) was also performed.

3. Results and Discussion

CV at several potential scan rates (Figure 1a) revealed more than 40% increase in electrochemical surface area for nITO electrodes with respect to tfITO. The $Fe(CN)_6^{3-/4-}$ oxidation current peak as a function of the square rooted potential scan rate is represented in Figure 1b, where the slope is directly related to the electrochemical surface area by the Randles-Sevcik relationship, shown in Equation (1), resulting 40% higher in the case of nITO:

$$I_{\mathrm{pa}} = \kappa n F A C \sqrt{\frac{nF\nu D}{RT}} \quad (1)$$

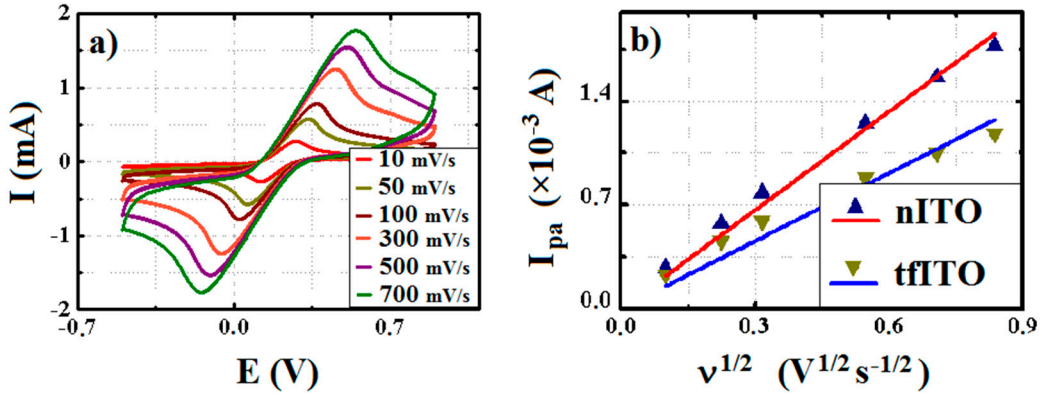

Figure 1. (**a**) Cyclic voltammetry at several potential scan rates for annealed nITO electrodes; (**b**) Linear representation of oxidation peak current vs. square rooted potential scan rate (ν), for annealed nanostructured (blue markers, red line) and thin (green markers, blue line) ITO films.

XPS and FTIR were used to confirm GPTMS layers formation (Figures 2a,b and 3a, respectively). The spectra show covalent silane bonding formation on the ITO surface. Then, a ferrocene-labeled organic molecule was coupled to the GPTMS-derivatized electrodes as a proof-of-concept. Ferrocene's redox activity on nITO was detected by CV (Figure 3b), showing an increase in the oxidation peak height of more than 400% compared to tfITO [7,8].

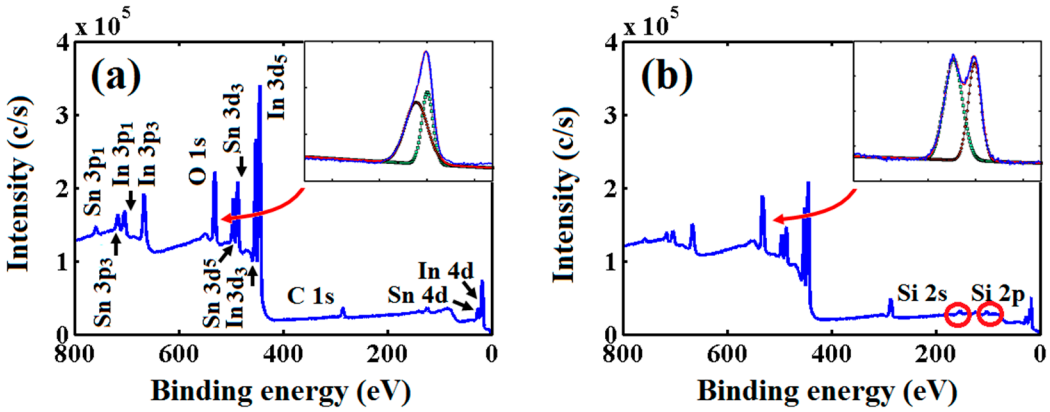

Figure 2. (**a**) XPS spectra of bare nITO films and (**b**) GPTMS-derivatized nITO films, where silicon peaks corresponding to GPTMS molecules can be observed. Insets show a separation in the oxygen peak as a consequence of GPTMS binding with superficial –OH radicals.

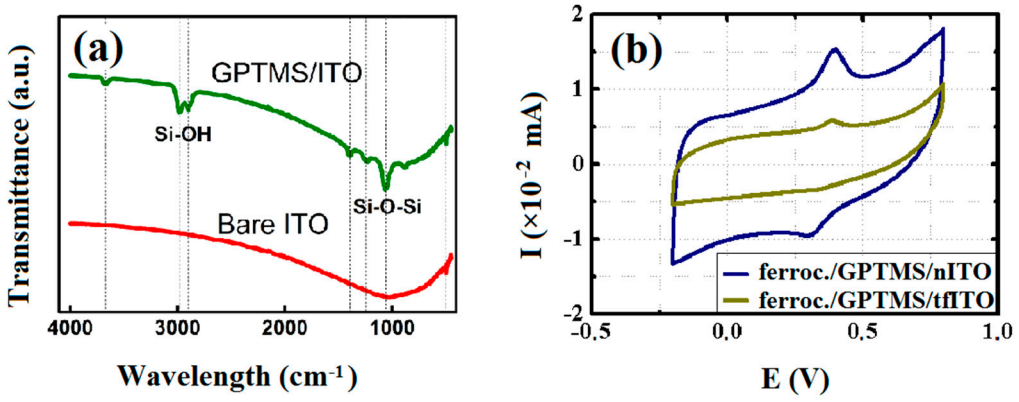

Figure 3. (**a**) Comparison of FTIR spectra for bare nITO films (red) and GPTMS-modified ITO surfaces (green); (**b**) Cyclic voltammetry at 50 mV/s for nanostructured (blue) and thin (green) ITO films showing the redox activity of ferrocene molecules immobilized on the GPTMS-derivatized electrodes.

4. Conclusions

In summary, the viability of nanostructured ITO films as electrodes for electrochemical sensors is reported, and microscopic (XPS and FTIR) and macroscopic (CV) evidences of the electrodes functionalization indicate a higher throughput compared to thin ITO films, opening the door to a wide range of biomedical and environmental applications. Future experiments will involve the performance of a full immunochemical assay on the ITO electrodes for the development and characterization of a real biosensor based on this material, and also the determination of the limit of detection, sensitivity and specificity.

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Conflicts of Interest: The authors declare no conflict of interest. The funding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

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