Development of individual semiconductor nanowire for bioelectrochemical device at low overpotential conditions

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Abstract
In this work we report the bioelectrochemical study using an individual indium tin oxide (ITO) nanowire (ITO-NW) electrode modified with glucose oxidase enzyme (GOx), in which the enzymatic activity and the biocatalytic activity was evaluated. The main objective is to show that at low overpotential condition, semiconductor NW can be used as an electron donor during biocatalytic process. We demonstrate the possibility of immobilizing an ITO-NW electrode on gold contacts deposited on top of a microchip (oxidized Si wafer). A protective polymer layer containing an aperture over the sample area was photolithographically deposited over the microchip to isolate the metallic contacts. For \( \text{H}_2\text{O}_2 \) reduction during the biocatalysis at ITO-NWs surface, with \( \eta \approx 50 \text{ mV} \), normal linear behavior is not observed and an exponential current is evident, similar to \( n-p \) semiconductor junction behavior. These results can open new tools for studying redox enzymes at the single-molecule level, and the device described here is very promising as a candidate for further exploration in bioelectrochemical devices, such as biofuel cells and biosensors.

1. Introduction

Recently, many efforts have been made to build electrochemical devices with geometry at the nanometer level, which provide new tools for electrochemical studies [1–4]. The use of individual 1D structure, such as single-walled carbon nanotubes (SWNTs) as electrodes for electrochemistry [1–3], has opened a differentiated route toward electrochemical biomolecule studies. Also, boron-doped silicon nanowires (SiNWs) can be used as sensitive biosensors, where nanosensors are capable of highly sensitive and selective real-time detection of proteins at different concentration levels [4]. In the same field of application, devices based on Sn doped In\(_2\)O\(_3\) nanowires (ITO-NWs) are interesting due to, by decreasing the dimensions of the wires, the electrochemical properties at the Fermi level can be tuned and the change from metallic to a nonmetallic character of the transport is evidenced [5]. Because of this, a mere interference at interface ITO-NWs/electrolyte causes a modification of the charge distribution and transport mechanism, and its can be used in the conversion of biological activity to an electrochemical signal.

In this communication we report the bioelectrochemical study using individual ITO-NW electrode modified with glucose oxidase enzyme (GOx), in which the enzymatic activity and the biocatalytic activity was evaluated. The main objective is to show that at low overpotential condition, semiconductor single NW can be use as an electron donor during the biocatalytic process. Also, we found that the current–potential law for an \( n-p \) semiconductor junction can be applied successively for this case.

2. Experimental

2.1. Sn doped In\(_2\)O\(_3\) nanowires synthesis and characterization

ITO nanowires were prepared by the carbothermal evaporation technique [6], where In\(_2\)O\(_3\) and SnO\(_2\) powders were mixed with 10% (weight) of carbon black. Each powder was placed inside of two separate alumina crucibles, aligned in the hot zone of a tube furnace with controlled temperature and gas flow. The experimental conditions were: 1150 °C, with \( \text{N}_2 \) gas atmosphere during 6 h. The resulting material presented a woolly appearance. The morphology of the nanowires was investigated by scanning transmission electron microscopy (STEM) in bright-field mode and by field-emission scanning electron microscopy (FE-SEM Zeiss, Model Supra 35).

2.2. Microchip fabrication

Microfabricated metal (Au/Ni, 100 nm) electrodes were prepared onto a thermally oxidized Si wafer with an oxide layer...
(SiO$_2$) of 300 nm thickness. The samples containing ITO-NW were ultrasonically dispersed in ethanol and were placed onto the metallic electrodes. An individual ITO-NW electrode on gold contacts was deposited atop of the microchip. A protective polymer layer containing an aperture over the electrode area was photolithographically deposited over the microchip, in order to isolate the metallic contacts, as shown in Fig. 1.

2.3. Enzyme immobilization

A layer of GOx was attached to the NW by drop coating. A total amount of 480 μL of solution of enzyme solution was used. The enzyme solution was previously prepared by adding 40 mg BSA and 100 mg GOx in 2 mL of 0.1 mol L$^{-1}$ phosphate buffer at pH 7.0. The ITO-NW electrodes were prepared by drop coating of a mixture of proteic solutions. The electrode was dried for 1 h at 22 °C.

2.4. Electrochemical measurements

Electrochemical experiments were performed in a two-electrode cell system. A gold electrode was used as a counter electrode, and ITO-NW or ITO-NW/glucose oxidase electrodes as the working electrode. The electrolytic solution (20 μL) was a phosphate buffer solution 0.1 mol L$^{-1}$, pH 7.0. Linear sweep voltammetry measurements were carried out using an electrochemical analyzer (Keithley Model 237). All measurements were performed at 22 ± 1 °C.

3. Results and discussion

Firstly, ITO-NWs were obtained by the carbothermal evaporation [5,6] process of In$_2$O$_3$ and SnO$_2$. An individual ITO-NW electrode on gold contacts was deposited on top of a microchip (oxidized Si wafer), as shown in Fig. 2a. We use distinct nanomanipulation procedures to be applied for ITO-NWs nanoelectrodes, in order to build-up an enzyme device with nanometric dimensions. A protective polymer layer containing an aperture over the electrode area was photolithographically deposited over the microchip, in order to isolate the metallic contacts (Fig. 1). GOx enzyme was deposited at ITO-NW electrode surface by drop coating [7,8] and the final configuration of the electrochemical cell is shown in Fig. 2b.

Several electrochemical measurements were conducted in order to observe the bioelectrochemical response of modified electrode in the presence of glucose. The electrochemical behavior at low overpotential condition of an ITO-NW/GOx modified electrode in the presence and in absence of glucose is shown in Fig. 3. In the presence of glucose, an increase of cathodic current is observed due to H$_2$O$_2$ reduction at ITO-NW surface, from biocatalytic conversion of glucose to H$_2$O$_2$. A scheme of enzymatic reaction is shown in Fig. 2b, which depicts overpotential and cathodic current densities after the enzymatic reaction. Two different electrode situations are depicted in Fig. 2b, in order to exemplify the current at an electrode/solution interface, according to the value of the overpotential, and showing the displacement from equilibrium of the electric potential of the electrode. For instance, the equilibrium referred to is that of some specific interfacial electron-transfer reaction, in this case cathodic reduction of hydrogen peroxide, as will be presented in the following.
On the other hand, when high currents more often than Tafel behavior. In Fig. 4, the dependence at the semiconductor/solution interface shows limiting the difficulty the electron to overcome the energy barrier due to the electric field applied. As a consequence, the current potential dependence at the semiconductor/solution interface shows limiting currents more often than Tafel behavior. In Fig. 4, the n-type electrode shows an exponential behavior on the cathodic profile for electron-rich electrode surface (0 < \eta < 50 mV). As expected, the concentration of electrons at the surface of a semiconductor depends on the applied overpotential.

Interesting points can be observed from these data. Firstly, considering metallic electrodes with macro size dimensions and with bulk properties, the electrochemical reduction of H\textsubscript{2}O\textsubscript{2} depends on the overpotential applied, and two reaction paths should be considered: the high overpotential and the low overpotential case. In both cases, the Butler–Volmer (B–V)\[9\] equation has been used to express the quantitative dependence of the electrochemical reaction rate on overpotential condition, as shown in Eq. (1).

\[
I = I_0\left(e^{\frac{\eta F}{RT}} - e^{\frac{1 - \eta F}{RT}}\right)
\]  

(1)

where \(I\) is electrode current (A), \(I_0\) is exchange current density, (A/m\textsuperscript{2}), \(\eta\) is the overpotential (V), \(T\) is absolute temperature (K), \(F\) is the Faraday constant, \(R\) is universal gas constant and \(\beta\) is symmetry factor.

In the case of a negative current (reduction of H\textsubscript{2}O\textsubscript{2}), electrons must flow from the electrode to the solution. In conventional electrochemical devices (bulk electrodes) and for low overpotential condition, \(\eta\) is numerically less than \(RT/\beta F (~50 mV)\) and a linear current density variation with the overpotential is expected by applying Eq. (1) and by using the Taylor–MacLaurin [9] expansion. On the other hand, when high \(\eta\) values are reached (>50 mV), the current rises exponentially.

In our experiments for H\textsubscript{2}O\textsubscript{2} reduction at ITO-NWs surface, with \(\eta \leq 50 mV\), the normal linear behavior is not observed and an exponential current is evident. This indicates that confined electrons at the Fermi level posses sufficient greater energy to process the electrochemical reaction, transforming H\textsubscript{2}O\textsubscript{2} in electrochemically reduced products.

Thus, the important question is: why is the current increase totally asymmetrical? In order to explain this phenomenon we have used the concept of the electrochemical current–potential law for a n–p semiconductor junction [9]. In this case, the electrons only flow from electrode if the hydrogen peroxide is similar to a p-like semiconductor at this junction. For an electron-transfer reaction from an n-type semiconductor to an acceptor, we can write:

\[
I = I_0\left(e^{\frac{-\eta F}{RT}}\right)
\]

(2)

Note that there is no symmetry factor \(\beta\) in the exponential (\(i\) vs. \(\eta\)), as opposed to B–V equation. In this case, we are considering the difficulty the electron to overcome the energy barrier due to the electric field applied. As a consequence, the current potential dependence at the semiconductor/solution interface shows limiting currents more often than Tafel behavior. In Fig. 4, the n-type electrode shows an exponential behavior on the cathodic profile for electron-rich electrode surface (0 < \(\eta < 50 mV\)).

4. Conclusions

In conclusion, a new approach to prepare an individual ITO-NW electrode modified with enzyme (GOx) was presented, in which the enzymatic activity and the biocatalytic activity were evaluated under low overpotential conditions. We found that current-potential for a single ITO-NW modified with GOx shows similar behavior to an n–p semiconductor junction. This result and the device build-up system is very promising as a candidate for further exploration in bioelectrochemical devices, such as biofuel cells [10,11] and biosensors [12–16]. Furthermore, this applied potential range ensures minimization of interference effects when the biodevice is used in real and complex matrices, such as biological media [7,16,17]. Also, these results can open new tools for studying redox enzymes at the single-molecule level, since that recent studies [18] has pointed that single-enzyme molecule bioelectrochemistry will become possible in the near future.

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References