

## Research Letter

# Gold and TiO<sub>2</sub> Nanostructured Surfaces for Assembling of Electrochemical Biosensors

**Antonella Curulli and Daniela Zane**

*CNR-ISMN, Istituto per lo Studio dei Materiali Nanostrutturati, Via del Castro Laurenziano 19,  
7 00161 Roma, Italy*

Correspondence should be addressed to Antonella Curulli, [antonella.curulli@uniroma1.it](mailto:antonella.curulli@uniroma1.it)

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Devices based on nanomaterials are emerging as a powerful and general class of ultrasensitive sensors for the direct detection of biological and chemical species. In this work, we report the preparation and the full characterization of nanomaterials such as gold nanowires and TiO<sub>2</sub> nanostructured films to be used for assembling of electrochemical biosensors. Gold nanowires were prepared by electroless deposition within the pores of polycarbonate particle track-etched membranes (PTMs). Glucose oxidase was deposited onto the nanowires using self-assembling monolayer as an anchor layer for the enzyme molecules. Finally, cyclic voltammetry was performed for different enzymes to test the applicability of gold nanowires as biosensors. Considering another interesting nanomaterial, the realization of functionalised TiO<sub>2</sub> thin films on Si substrates for the immobilization of enzymes is reported. Glucose oxidase and horseradish peroxidase immobilized onto TiO<sub>2</sub>-based nanostructured surfaces exhibited a pair of well-defined and quasireversible voltammetric peaks. The electron exchange between the enzyme and the electrodes was greatly enhanced in the TiO<sub>2</sub> nanostructured environment. The electrocatalytic activity of HRP and GOD embedded in TiO<sub>2</sub> electrodes toward H<sub>2</sub>O<sub>2</sub> and glucose, respectively, may have a potential perspective in the fabrication of third-generation biosensors based on direct electrochemistry of enzymes.

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## 1. INTRODUCTION

The use of nanoscale materials for electrochemical biosensors has seen explosive growth in the past 5 years, since discovery of low-potential detection of NADH on carbon nanotube-modified electrode by Musameh et al. [1] and the first use of gold nanoparticles as labels for the electrochemical immunosensors by Dequaire et al. [2]. Many research papers and general reviews using nanomaterials for electrochemical biosensing [3, 4] have been published since then.

In this work, we report the characterization of nanomaterials such as gold nanowires and TiO<sub>2</sub> nanomaterials to be used for assembling of electrochemical biosensors and the comparison between the two systems used. The immobilization of single or clustered molecules of enzymes on the nanostructured surfaces could be an important step towards the development of a new class of biosensors.

## 2. EXPERIMENTAL

Further details of the experimental part have been mentioned in the Supplementary Material (available online at doi:10.1155/2008/789153).

The chemical analysis (X-ray photoelectron spectroscopy) was performed using an Escalab Mk II spectrometer, equipped with a standard Al K $\alpha$  excitation source ( $h\nu = 1486.6$  eV) and a five-channeltron detection system.

The FT-IR spectra were recorded with an IR Scope II Bruker, Interferometer Equinox 55 (Bruker).

The electrochemical measurements were performed at room temperature in a conventional one-compartment cell with a three-electrode configuration using an Autolab PG-STAT 12 potentiostat/galvanostat (Eco Chemie, the Netherlands). The reference electrode was Ag/AgCl and a Pt electrode was the counter.

TABLE 1: Electrochemical characterization of gold nanowires-modified electrodes with SAM (MPA in KCl 0.2 M).  $I_{pa}$  is the peak current at gold nanowires SAM-modified electrode,  $I_o$  is the peak current for non-nanostructured electrode.

	$E_{pa}$ (V)		$I_{pa}$ ( $\mu$ A)		$I_{pa}/I_o$
	Au/MPA	Nano-Au/MPA	Au/MPA	Nano-Au/MPA	
$Fe(CN)_6^{3-}$	0.27	0.36	13.83	210.64	15.23
$Ru(NH_3)_6^{3+}$	-0.11	-0.33	20.42	200.00	9.79

TABLE 2: Electrochemical characterization of gold nanowires-modified electrodes with SAM (MPE in KCl 0.2 M).  $I_{pa}$  is the peak current at gold nanowires SAM-modified electrode,  $I_o$  is the peak current for non-nanostructured electrode.

	$E_{pa}$ (V)		$I_{pa}$ ( $\mu$ A)		$I_{pa}/I_o$
	Au/MPE	Nano-Au/MPE	Au/MPE	Nano-Au/MPE	
$Fe(CN)_6^{3-}$	0.28	0.35	18.14	187.76	10.35
$Ru(NH_3)_6^{3+}$	-0.10	-0.04	21.70	252.13	11.62

### 3. RESULTS AND DISCUSSION

#### 3.1. Gold nanowires

The synthesis and morphological characterization of gold nanowires have been discussed elsewhere [5]. In this work, we focused on nanowires grown with a synthesis time of 24 hours.

After the preparation of the nanowires film, the next steps towards the biosensor fabrication are the SAM formation and subsequent activation to allow the attachment of the enzyme. The resulted nanowires system should allow the diffusion and the immobilization of the GOD, a large enzyme [6]. Hou et al. [7] demonstrated that the films prepared by electroless deposition can be used as substrates to support densely packed SAMs formed from long chains. In this paper, short-chain alkane thiols have been used because the long-chain systems have the drawback of a great distance of the enzyme redox centre from the electrode surface and of a limited diffusion.

3-mercaptopropionic acid (MPA) and 2-mercaptoethylamine (MPE) were used to link the enzyme onto the surface. The formation of SAMs on electroless has been qualitatively assessed by measurements of contact angles of water. For bare electroless gold, a contact angle of  $75 \pm 8^\circ$  was found. This value decreases to  $32 \pm 3^\circ$  for Nano-Au/MPE and to less than  $10^\circ$  for Nano-Au/MPA. This modification of the contact angles is, therefore, a useful proof of the presence of hydrophilic at the surface of electroless gold [8].

The effective surface area of electrochemically active gold supporting SAM was estimated by cyclic voltammetry in 0.1 M  $H_2SO_4$ . The Nano-Au/MPE system has a fractional coverage of 74% and the Nano-Au/MPA has a coverage of 55% according to the literature data [9].

In Tables 1 and 2, the values of the peak current for nanostructured and non-nanostructured systems are reported, named, respectively,  $I_{pa}$  and  $I_o$ . We observed a ratio between the peak current in the presence and in the absence of the nanostructured film ranging from 9.79 up to 15.23,

which indicates an increase of the electrochemical response using the nanowired system. Similar data were obtained for the other systems, not shown here for brevity.

#### *Enzyme immobilization and electrochemical detection of glucose*

In the system modified with  $-NH_2$  terminal thiol (Nano-Au/MPE), the enzyme molecules were covalently attached using glutaraldehyde as linking agent, while the covalent attachment of GOD to the carboxylic-terminated SAM was achieved via carbodiimide activation (EDC + NHS). In this case, the physical adsorption immobilization failed the stability test during time: in fact, in our experimental conditions, the biosensors obtained have worked only for few hours. In any case, generally, the physical adsorption has not been used as enzyme-immobilizing system for SAM-modified surface [8, 9].

The different enzyme electrodes have then been used to detect the target analyte (glucose) by cyclic voltammetry ranging the analyte concentrations from 1 to 6 mM.

The highest current response was seen for the Nano-Au/MPE/GOD system and the corresponding regression linear equation is the following:  $y = 68.629x + 28.61$ , where  $y$  represents the current in  $\mu$ A and  $x$  the glucose concentration in mM,  $R^2$  was 0.9987. This high response could be due to the higher roughness of the nanostructured surface compared to a flat gold electrode and to the tubular nature of the electrode, both leading to an increased surface area of the electrode for the same geometric area [10]. Another possible explanation of the high sensitivity observed with our system could be that the amount of the active enzyme molecules is higher when they are immobilized within porous system than on a flat surface. The difference observed between the two immobilized systems is probably due to the fact that the glutaraldehyde system probably loads a lower quantity of the enzyme because the linker agent is largely diluted before being used.

### 3.2. TiO<sub>2</sub>-based nanomaterials

The morphological characterization of TiO<sub>2</sub>-modified electrodes was discussed elsewhere [11, 12].

The enzymes have been immobilized by physical adsorption because the functionalised surface should be an optimal platform for anchoring enzymes. The effectiveness of enzyme immobilization was determined by XPS and FT-IR measurements, performed on TiO<sub>2</sub>-GOD- and TiO<sub>2</sub>-HRP-modified electrodes. From XPS data, three components (C1, C2, and C3) were observed, corresponding, respectively, to the aliphatic bond CH-CH present in GOD structure, and to the different carbon atoms of the characteristic amidic groups [13]. The presence of adsorbed GOD enzyme on TiO<sub>2</sub>-modified Si electrode was confirmed by FT-IR spectra, showing the characteristic amide I band (1649 cm<sup>-1</sup>) due to the stretching vibration of peptide C = O groups, and amide II band (1556 cm<sup>-1</sup>), which results from a combination of N-H in-plane bending and C-N stretching of peptide groups [14]. We observed only a slight shift with respect to the amide peak positions of GOD powder so that the immobilization process used retains the native GOD conformation [15], but we could suggest a strong interaction between the enzyme and the surface just to justify the shift of the amide peak position. Similar results (not shown for the sake of brevity) were obtained for the immobilized HRP enzyme.

As shown in Figure 1, direct electron transfer of GOD and HRP at TiO<sub>2</sub>-modified Si electrodes was observed. The redox peaks potentials are consistent with reported values for free FAD and FAD redox centre for flavoenzyme [16]. The peak positions remain constant as the scan rate increases, while the redox peak currents are proportional to the scan rate in the range less than 100 mV/s, indicating a typical surface-controlled quasireversible process [17].

The amperometric response of GOD and HRP third-generation biosensor based on functionalised TiO<sub>2</sub>-Si electrodes are shown in Figure 2. The calibration curve for glucose (Figure 2(a)) and for H<sub>2</sub>O<sub>2</sub> (Figure 2(b)) showed a linear response for the biosensor in the concentration range of  $5.0 \times 10^{-6}$  to  $5.5 \times 10^{-4}$  M for glucose and  $1.0 \times 10^{-6}$  to  $2.0 \times 10^{-4}$  M for HRP, with a time response of few seconds (7 seconds for GOD and 6 seconds for HRP) for successive enzyme injection. At higher glucose and H<sub>2</sub>O<sub>2</sub> concentrations, the calibration curves tend to level off, showing a typical Michaelis-Menten mechanism. The apparent Michaelis-Menten constant (K<sub>m</sub>) was calculated at different enzyme concentration. Small K<sub>m</sub> values were obtained for 5 mg/mL of GOD (K<sub>m</sub> = 7.5 mM) and HRP (K<sub>m</sub> = 1.0 mM) concentrations, indicating a great affinity between enzyme and electrode surface. Decreasing the enzyme concentration, K<sub>m</sub> values increase up to, respectively, 30 mM and 5.8 mM for 1 mg/mL of GOD and HRP concentration, indicating that glucose and H<sub>2</sub>O<sub>2</sub> biosensors still work well at low enzyme concentration.

## 4. CONCLUSIONS

Glucose oxidase was immobilized on the nanostructured gold-modified surfaces via self-assembling monolayers. Elec-

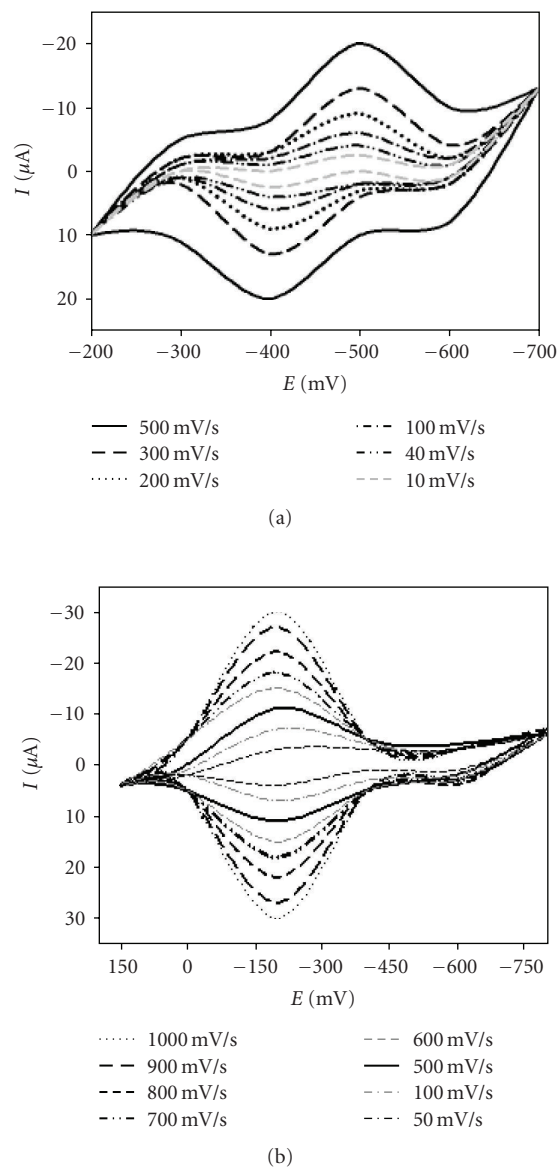


FIGURE 1: Cyclic voltammograms for (a) GOD-TiO<sub>2</sub>-modified Si electrodes, (b) HRP-TiO<sub>2</sub>-modified Si electrodes at different scan rate and at pH 7.0 in 0.1 M phosphate buffer solution.

trochemical analysis showed that a great enhancement of the peak current occur if the sensitive material is deposited on a nanostructured surface, which makes gold nanowired surfaces excellent candidates for biosensing applications, but further work should be necessary to improve the stability of our system.

On the other hand, nanostructured TiO<sub>2</sub>-based layers provide an efficient immobilization of HRP and GOD monolayers on Si platforms, without the use of any mediators, considering the literature data for other glucose electrochemical sensors [18], the obtained results resulted very promising. The direct electron transfer between enzyme and electrodes is enhanced by the nanostructured environment of TiO<sub>2</sub>-based layers. Amperometric glucose and H<sub>2</sub>O<sub>2</sub>

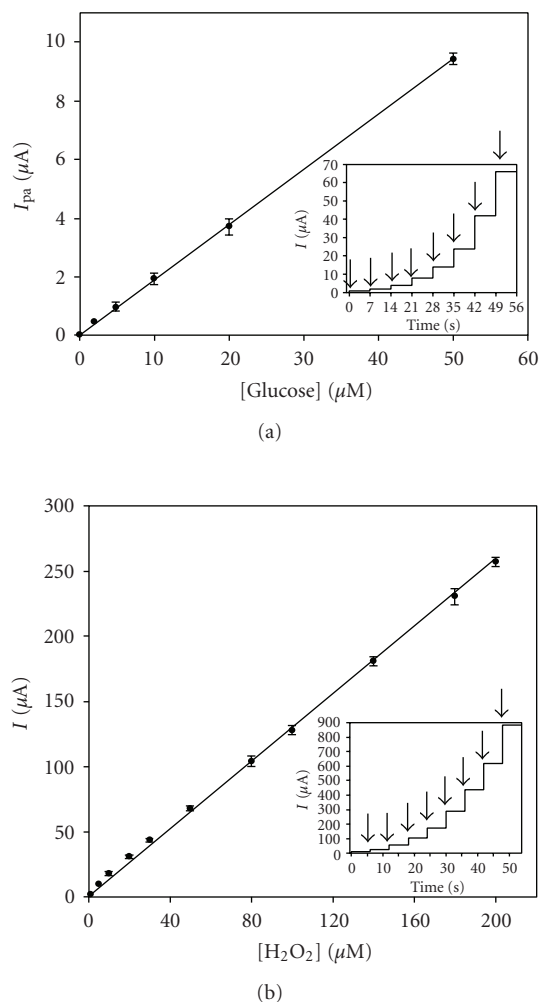


FIGURE 2: Calibration curve of (a) glucose biosensor based on  $TiO_2$ -modified Si electrodes, and (b)  $H_2O_2$  biosensor based on  $TiO_2$ -modified Si electrodes. Inset graphs show typical amperometric response of the biosensors to successive injection of glucose and  $H_2O_2$  into stirring 0.1 M phosphate buffer solution at pH 7.0.

third-generation biosensors based on the  $TiO_2$ -modified Si-electrodes showed good sensitivity, low detection limit ( $10^{-6}$  M), and fast time response (few seconds).

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