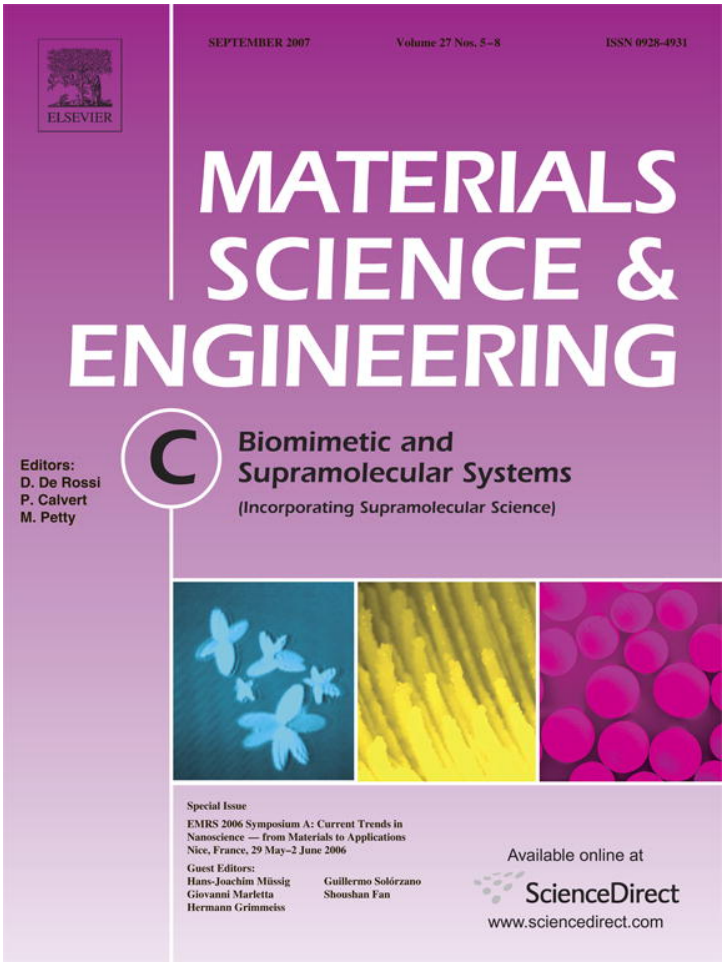

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Feasibility of enzyme biosensors based on gold nanowires

A. Cusmà [a,*], A. Curulli [b], D. Zane [b], S. Kaciulis [a], G. Padeletti [a]

[a] *Institute for the Study of Nanostructured Materials (ISMN)-CNR, Montelibretti Research Division, Via Salaria Km 29,300, 00016 Monterotondo (Rome), Italy*
[b] *Institute for the Study of Nanostructured Materials (ISMN)-CNR, Rome 2 Research Division, Via del Castro Laurenziano 7, 00161 Rome, Italy*

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Abstract

Gold nanowires were prepared by electroless deposition within the pores of polycarbonate particle track-etched membranes (PTM). Glucose oxidase was deposited onto the nanowires using self-assembling monolayer as anchor layers for the enzyme molecules. AFM characterization was performed before and after the immobilization of the enzyme. Finally cyclic voltammetry was performed for different enzymes to test the applicability of gold nanowires as biosensors. The AFM measurements proved the immobilization of the enzyme while the electrochemical characterization showed that the presence of nanowired substrates greatly enhances the electrical response of the biosensor.

Keywords: Gold nanowires; Template synthesis; Electroless plating-deposition; Atomic Force Microscopy; Biosensors

1. Introduction

The development of reliable electrochemical biosensors is a field of great importance in today's technology since they are very promising to replace a wide range of analytical techniques, thanks to their unique properties and characteristics [1–4].

A crucial problem in the commercial development of such biosensors is the ability to immobilize an enzyme on an electrode without any loss of its biological activity. Many methods have been proposed to overcome this problem, among which the use of self-assembling monolayer as anchor layers for the enzyme molecules has been extensively studied [5–8]. However, if monolayers are used, only a very small amount of enzyme is immobilized and this will turn to a very low amperometric response of the biosensor. On the contrary, the use of gold nanowires as electrodes provides a huge surface area per unit volume allowing the immobilization of a larger amount of enzyme.

In this work we report the preparation and characterization of gold nanowire arrays to be used as a starting point for the realization of electrochemical biosensors.

Gold nanowires were prepared by electroless deposition of the metal within the pores of polycarbonate particle track-etched membranes, and given a complete chemical and morphological characterization both before and after the immobilization of GOD enzyme (glucose oxidase) via self-assembling monolayers. Then cyclic voltammetry was performed to test the applicability of gold nanowires as biosensors.

2. Experimental

2.1. Materials

Most of the materials and solutions used have been described in a previous work [9]. In addition, GOD from *Aspergillus niger* (E.C. 1.1.3.4, type VII-S), 3-mercaptopropionic acid (MPA, 99%), 2-mercaptoethylamine (MPE, cysteamine, >98%), glutaraldehyde (GA, 25%, in water), D-glucose, *N*-hydroxysuccinimide (NHS), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), 2-(*N*-morpholino)ethanesulphonic acid (MES), potassium ferrycyanide and hexammineruthenium (III) chloride were used as received from Sigma-Aldrich.

2.1.1. Gold nanowires preparation and electrochemical characterization

Electroless metal deposition involves the use of a chemical reducing agent to plate a metal from a solution onto a surface and the nanowires were synthesized according to the literature method [9].

* Corresponding author.
E-mail address: antonio.cusma@mlib.cnr.it (A. Cusmà).

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The formation of SAM was accomplished by adsorption of the thiols on the nanostructured gold surface: the substrates were immersed for 17 h in 2×10^{-3} M of MPA or MPE using ethanol as a solvent. The samples were then rinsed with ethanol and dried under a nitrogen stream.

The SAM modified membranes were electrochemically characterized by cyclic voltammetry at a scan rate 0.02 V s^{-1} using potassium ferricyanide 0.01 M and hexammineruthenium (III) chloride 0.01 M solutions in KCl 0.2 M, and the obtained results were compared with those of a SAM modified electrode.

2.1.2. Immobilization of GOD

The terminal carboxylic acid groups of MPA modified gold surfaces were activated by immersion in a pH 3.5 MES buffer solution containing 2 mM EDC and 5 mM NHS for 2 h. The surface was rinsed with the buffer solution and immediately placed in a solution of GOD in a phosphate buffer of 0.1 M.

Electrodes were incubated for 2 h in 1 mg ml^{-1} GOD solution.

The self-assembled cysteamine monolayers were soaked for 2 h in a solution of glutaraldehyde (commercial solution diluted 100-times in PBS) at room temperature.

The resulting monolayers were rinsed with buffer and placed for 2 h in a solution of GOD in phosphate buffer. Enzyme electrodes were rinsed with PBS and used immediately or stored at 4 °C in buffer solution.

2.1.3. Morphological characterization

Atomic Force Microscopy (AFM) analysis have been performed in air with a Dimension 3100 Digital Instruments microscope. Silicon cantilevers and tips have been used and the choice was made to work in tapping mode. With such technique, there is no contact between the tip and sample and so there is no possibility of damage to the sample surface and the degradation of the tip is reduced. Topographic images have been captured over scanning areas of various dimensions (1×1 μm^2 to 30×30 μm^2) so that an extensive characterization of the surfaces has been made possible.

2.1.4. Electroanalytical procedure for the detection of glucose

The electrochemical measurements were performed at room temperature in a conventional one-compartment cell with a three-electrode configuration using an Autolab PGSTAT 12 potentiostat/galvanostat (Ecochemie Netherlands). The reference electrode was Ag/AgCl and a Pt electrode was the counter. The gold membrane modified electrode, used as a working electrode, was placed between an Au disk and an O-ring joint. The O-ring determined the area serving as bioelectrode. For the electrochemical measurements of glucose, the enzyme electrodes were placed in a pH 7 buffer solution and the additions of glucose were made from a stock solution of glucose 1 M in PBS pH 7.2. The glucose solution was allowed to mutarotate for at least 24 h, before use.

Table 1
Electrochemical characterization of gold nanowires modified electrodes with SAM (MPA in KCl 0.2 M)

	E_{pa} (V)		I_{pa} (μA)		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

I_{pa} is the peak current at gold nanowires SAM modified electrode, and I_o is the peak current for non-nanostructured electrode.

Table 2
Electrochemical characterization of gold nanowires modified electrodes with SAM (MPE in KCl 0.2 M)

	E_{pa} (V)		I_{pa} (μA)		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

I_{pa} is the peak current at gold nanowires SAM modified electrode, and I_o is the peak current for non-nanostructured electrode.

3. Results and discussion

3.1. Electrochemical characterization of gold nanowires modified electrodes

The preparation and characterization of gold nanowires have been discussed elsewhere [9], as well as the different steps of the nanowires growth process and the influence of the growth time on the nanowires properties. In this work we focused on nanowires grown with a synthesis time of 24 h.

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 [10]. Hou et al. [11] demonstrated that the films prepared by electroless deposition can be used as substrates to support the densely packed SAMs formed from long chains. In this work, short chain alkane thiols have been used because the long chains 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 two systems were electrochemical characterized by cyclic voltammetry in KCl 0.2 M, using $Fe(CN)_6^{-3}$ 0.01 M and $Ru(NH_3)_6^{3+}$ 0.01 M as electrochemical probes.

The formation of SAMs on electroless has been qualitatively assessed by measurements of contact angles of water. Advancing type contact angles of water were measured on gold surfaces modified and unmodified with alkane thiols at room temperature, using the sessile drop technique and an analysis system.

Table 3
Glucose calibration (responses) for modified electrodes

Glucose (mM)	I_{pa} (μA)			
	Au/MPE/GOD	Nano-Au/MPE/GOD	Au/MPA/GOD	Nano-Au/MPA/GOD
1	3.27	84.04	1.16	12.2
2	6.57	173.40	2.24	20.10
3	9.45	230.11	3.21	33.31
4	12.96	317.45	4.27	44.64
5	15.72	392.13	5.18	56.13
6	18.02	415.74	6.16	66.22

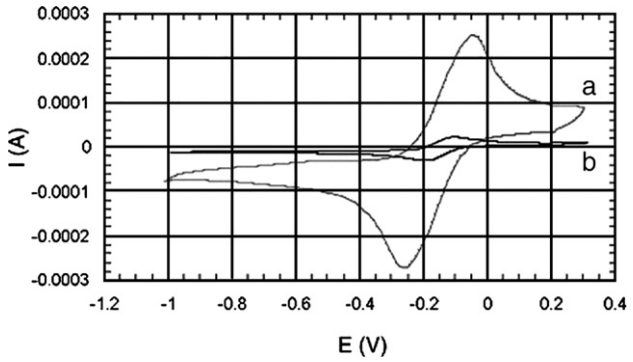

Fig. 1. Cyclic voltammetry of the MPE–GOD system on nanostructured (a) and non-nanostructured (b) surfaces, relative to the Ru probe.

For bare electroless gold, a contact angle of 75±8° was found. This value decreases to 32±3° for Nano-Au/MPE and to less than 10° 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 [12].

The effective surface area of electrochemically active gold supporting SAM was estimated by cyclic voltammetry in 0.1 M H_2SO_4. Fractional coverages were found from the decrease of the cathodic peak in the presence and in the absence of SAMs. 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 [13].

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. This is also well shown in Fig. 1, relative to the MPE/GOD system and the Ru probe. Similar graphics were obtained for the other systems, not shown here for brevity.

3.2. Chemical and morphological characterization

An XPS characterization, published elsewhere [14] confirmed the presence of the GOD enzyme on the surface of the gold modified samples.

AFM images were performed on the gold modified surface both before and after the deposition of the GOD enzyme (Fig. 2). The images shown here, taken on a 1×1 μm area, revealed the presence of the gold nanowires, oriented mostly perpendicularly to the surface (even if a number of them is parallel to the surface), whose lateral dimension goes from 10 to 50 nm. The image taken after the enzyme deposition shows the presence of the large agglomerate of enzyme molecules above the gold surface, which greatly enhances the surface roughness. The values of average roughness increase from 45.4 to 65.6 nm after the enzyme immobilization, while the values of the peak-valley mean excursion increase from 647.5 nm up to 870.9 nm.

3.3. 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 the linking agent, while the covalent attachment of GOD to the carboxylic terminated SAM was achieved via carbodiimide activation (EDC+NHS).

The different enzyme electrodes have then been used to detect the target analyte (glucose) by cyclic voltammetry. The highest current response was seen for the Nano-Au/MPE/GOD system (Table 3).

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 [15]. 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

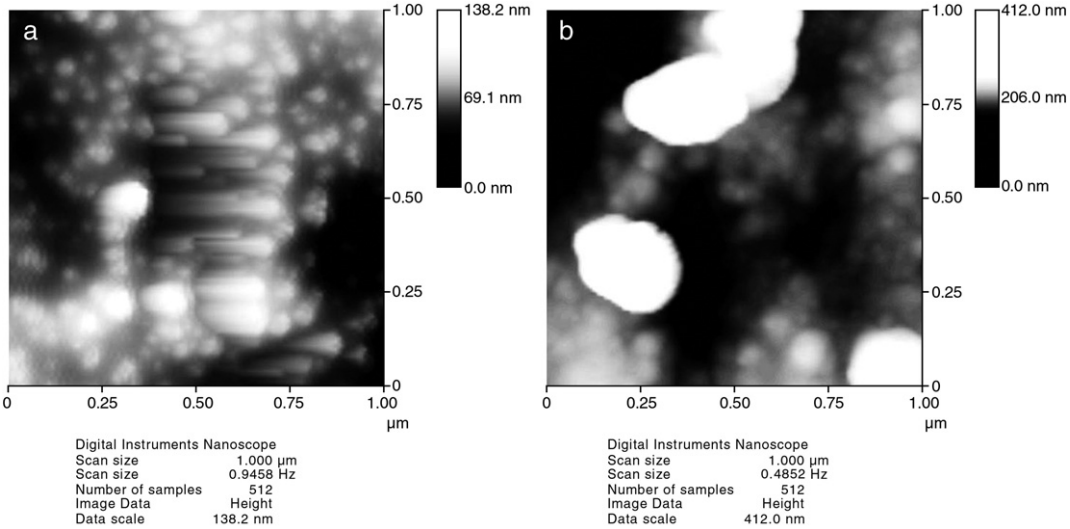

Fig. 2. 1×1 mm AFM micrographs of gold nanowired surface before (a) and after (b) the immobilization of the glucose oxidase.

the 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.

4. Conclusions

Gold nanowires were synthesized using the PTM method on polycarbonate membranes. Glucose oxidase was immobilized on the nanostructured gold modified surfaces via self-assembling monolayers. Chemical and morphological characterization confirmed the presence of both nanostructured gold arrays and immobilized GOD enzyme on the surfaces. Electrochemical analysis showed that a great enhancement of the peak current occurs if the sensitive material is deposited on a nanostructured surface, which makes gold nanowired surfaces excellent candidates for biosensing applications.

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