Highly Sensitive electrodic materials based on Pt Nanoflowers grown on Pt Nanospheres for Biosensor development

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Abstract— In this work we describe the realization of monometallic nanostructures by two simple electrodeposition steps. The surface of the modified electrode was characterized by scanning electron microscopy that confirms a homogeneous deposition of Pt nanospheres decorated with Pt nanoflowers. The so obtained nanostructured sensor exhibited good amperometric response towards hydrogen peroxide sensing at +0.30 V *vs* Ag|AgCl|Cl⁻. Therefore, it is especially useful toward glucose detection with reduced interferences. Glucose oxidase was immobilized onto the nanostructured surface by cross-linking with glutaraldehyde and the biosensor was characterized by chronoamperometric method in phosphate buffer. The biosensor showed a sensitivity of 29 ± 2 μA/(mM cm^2). Measurements in cell media reveal that the Pt-Pt hybrid nanostructures are promising for real-time glucose monitoring in real biosample.

Keywords—Pt nanospheres, Pt nanoflowers, hybrid nanostructures, H₂O₂ oxidation potential, cell media

I. INTRODUCTION

The field of electrochemical biosensors have received more attention in recent years thanks to some advantages such as low cost, high selectivity and simplicity in the realization. Different aspects must be considered to develop electrochemical biosensors, which are ideal for mass production. Firstly, the enzyme immobilized onto the electrode surface should retain a high affinity toward their substrate. Second, the realized biosensor must be easy and fast to prepare. At the same time, it should have high analytical performance [1,2]. As electrode substrates, transition metals have always attracted great attention thanks to their catalytic activity in different reactions. Platinum (Pt) is the most noble metal utilized as sensing electrode in biosensor field. It is largely employed because of its excellent performance toward the hydrogen peroxide (H_2O_2) detection that is the product of several reactions catalyzed by oxidase enzymes [3,4]. With the advent of nanotechnology, the research in the field of nanomaterials is increased thanks to their unique properties. Pt nanostructures have been used for the realization of more sensitive biosensors thank to their capability to enhance the catalytic activity toward H_2O_2 with respect the bulk metal [5]. The size of the nanostructures plays an important role because a smaller dimension usually results in higher catalytic properties [6,7]. Though Pt is a very used as catalyst, the most important drawback in building first generation biosensors (i.e. based on H_2O_2 detection) is that a high potential is required to obtain an amperometric response. Several other molecules, always present in biomedical samples, oxidize too at high potentials. This typically affects the biosensors specificity and increase. Moreover, high working potentials mean high energy consumption. Substrate contamination by adsorbed molecules on the flat surface is another disadvantage usually met [8,9]. To overcome these problems, the development of hybrid nanomaterials have attracted high interest for the combination of the properties of each component that exhibit better catalytic performance than the single component [10]. Different strategies are present in literature to obtain nanostructures. Among them, electrodeposition method is the simplest and fastest technique to obtain Pt nanomaterials free of contaminants. Moreover the metal loading could be easily controlled and it is possible to obtain nanomaterials with different shape and size. [11]. To our best knowledge, no hybrid monometallic nanostructures are present in literature so far. In this work, we report a novel Pt-Pt monometallic hybrid nanocomposite consisting in Pt nanospheres decorated with Pt nanoflowers ($Pt_{Ns}Pt_{Nf}$) and for the first time realized in our very recent [12] for applications to biosensing. The realization of this novel structure requires only two simple steps by electrodeposition in acidic solution. A first step is necessary to obtain a layer of Pt nanospheres while the second is needed to decorate them with Pt nanoflowers. With respect to the literature, our method present the advantage that does no require any template to obtain the different nanostructures. Avoiding the step to remove the template allow the final structures to be free of contaminants. The morphological surface of the obtained structures is here investigated by scanning electron microscopy (SEM) while the electrochemical characterization was carried out with a chronoamperometric method. Glucose oxidase was utilized as a model of oxidases and was immobilized onto the nanostructured surface by cross-linking with glutaraldehyde [13]. The monitoring of different metabolites such as glucose

produced from cell culture is very important in biomedical field [14]. For this reason, we performed chronoamperometric experiments also in a cell media.

II. MATERIALS AND METHODS

A. Chemicals

Chloroplatinic acid hexahydrate (H_2PtCl_6 $6H_2O$), H_2SO_4 (95-98 %), D-(+)-Glucose, Glutaraldehyde solution, Grade II, 25 % were purchased from Sigma. Tetrachloroplatinic acid (K_2PtCl_4) was purchased from Fisher-Sci. Glucose oxidase (GOx) grade I, 2 MU/5.06 g was obtained from Roche Diagnostics. Dulbecco's Modified Eagle's Medium was purchased from Sigma. Phosphate Buffer Saline (PBS) from Sigma at pH 7.4 was used to prepare the solutions.

B. Electrodeposition of Pt nanoflowers onto Pt nanospheres

Before electrodeposition, a Pt surface was cleaned in a 100 mM H_2SO_4 by applying a potential of +2.0 V *vs* Ag pseudoreference for 60 s [15]. Pt nanospheres were electrodeposited onto Pt surface using a three-electrode configuration with Pt as working electrode, carbon as counter electrode and Ag pseudoreference. A solution consisting of 25 mM K_2PtCl_4 and 50 mM H_2SO_4 was used for electrodeposition by applying a potential of -0.2 V *vs* Ag pseudoreference for 200 s. The modified electrode was cleaned in H_2SO_4 [15]. Pt nanoflowers were electrodeposited onto Pt nanospheres using a solution consisting of 25 mM H_2PtCl_6 and 50 mM H_2SO_4 and applying a potential of -1.0 V *vs* Ag pseudoreference. The as-prepared monometallic hybrid nanostructure was cleaned in H_2SO_4 as reported before [15].

C. Surface characterization

After electrodeposition, the morphological surface of the so obtained Pt nanoflowers onto Pt nanospheres was characterized by SEM by using a Zeiss Merlin Microscope (voltage 5kV).

D. Biosensor assembly

GOx was immobilized onto $Pt_{Ns}Pt_{Nf}$ surface by cross-linking with glutaraldehyde. 10 µl of a PBS solution (10 mM) containing glutaraldehyde 2.5 % and GOx (15mg/ml) was spread on the working of $Pt_{Ns}Pt_{Nf}$ electrode. The biosensors were kept overnight at 4°C before the experiments.

E. Electrochemical measurements

To perform chronoamperometric measurements an Autolab potentiostat with NOVA software (Metrohm Netherlands) and a conventional three-electrode configuration was utilized. Screen-Printed Electrodes (SPEs) (Metrohm, Switzerland) with a platinum working electrode (4 mm diameter), carbon counter electrode and Ag as reference electrode were used. Chronoamperometry experiments were carried out under stirring conditions with a successive addition of H_2O_2 or glucose to the cell by applying a fixed potential. All the applied potentials were referred to Ag│AgCl|Cl⁻ reference electrode. All the experiments and the electrodeposition were carried out under aerobic conditions at room temperature.

III. RESULTS AND DISCUSSION

A. Morphological characterization

In Fig.1, SEM image of the obtained Pt hybrid nanostructure is reported. As observed, the hybrid nanostructure consists of Pt nanospheres that were obtained during the first deposition with K_2PtCl_4, and of Pt nanoflowers obtained after the second deposition with H_2PtCl_6.
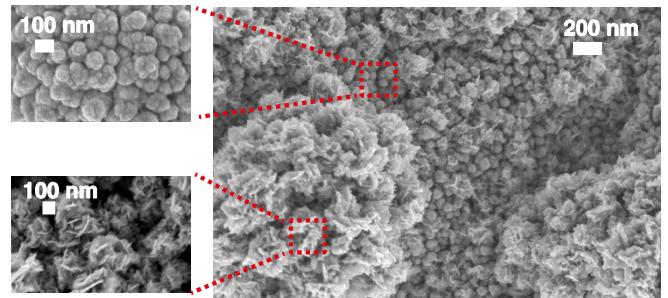

Fig.1. SEM image of Pt nanoflowers onto Pt nanospheres. Magnifications of Pt nanospheres (top left) and Pt nanoflowers (bottom left).

B. Influence of applied potential on amperometric response

Since glucose is usually indirectly determined by quantifying H_2O_2 generated by an enzymatic reaction [16], the amperometric response of H_2O_2 on $Pt_{Ns}Pt_{Nf}$ was investigated. In Fig. 2 the potential dependency of the current response obtained with Pt bare and $Pt_{Ns}Pt_{Nf}$ on the H_2O_2 detection (20 µM) is reported.
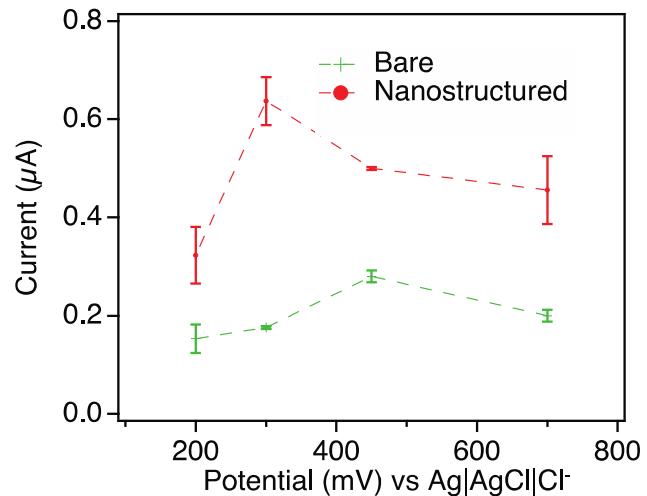

Fig .2. Effect of applied potential on the amperometric response at bare (green) and at $Pt_{Ns}Pt_{Nf}$ (red) electrode towards the detection of 20 µM H_2O_2.

As observed, the highest response is obtained at +0.45 V at Pt bare while with $Pt_{Ns}Pt_{Nf}$ the higher current is obtained at +0.30 V. These results suggest that the nanostructuration not only increases the electrochemical surface area and so the current response for a given applied potential but also lowers the detection potential that means reduced interferences and low power sensing. For these reasons, $Pt_{Ns}Pt_{Nf}$ was utilized to

assembly the biosensor and a potential of +0.30 V was selected for the further electrochemical characterization.

C. Amperometric response of the H_2O_2 sensor

The new $Pt_{Ns}Pt_{Nf}$ modified electrode was tested as H_2O_2 sensor using chronoamperometry mode at fixed potential +0.30 *vs* Ag│AgCl|Cl⁻ in PBS and cell media. The measurements were performed by successive addition of H_2O_2 in the concentrations range 0.05 – 0.25 mM, firstly in stirred PBS and then in cell media. The sensitivity of the sensor is twofolded in PBS as compared with that in cell media (119.7 and 47.5 µA/(mM cm²) in PBS and media, respectively). The value of sensitivity obtained in cell media is lower than that obtained in PBS indicating that the amperometric response decreases in the complex matrix. However, the response in cell media is reduced but not canceled.

D. Biosensor performance in PBS

The amperometric performance of the biosensor to glucose was investigated by chronoamperometry at fixed potential +0.30 V *vs* Ag│AgCl|Cl⁻ in PBS and in cell media and the chronoamperometry is reported in Fig.3 (a). Note that the used potential at +0.30 V was selected as working potential that is lower than the potential commonly utilized for first generation biosensors thanks to the presence of Pt nanostructures that reduce such working potential. In Fig. 3 (b), the amperometric response of the GOx biosensor as a function of glucose concentration in PBS and cell media is illustrated. By performing measurements in PBS, the biosensor showed a linear response in the range of 0.02 - 4.00 mM with a sensitivity of 29 ± 2 µA/(mM cm²) and a limit of detection of 6.6 ± 1.5 µM (n=3). The value of I_{max} obtained in PBS was 193 ± 10 µA/cm² (n=3). The apparent Michaelis-Menten constant (K_m^{app}), a parameter indicating the enzymatic affinity towards the substrate, was 3.9 ± 0.3 mM (n=3). This value of K_m^{app} is lower than those obtained with GOx in solution [5] and means that the Pt hybrid nanostructure is a good environment for the enzyme to maintain the native structure and to have high affinity toward glucose.

In Table 1, a comparison of the kinetic and electroanalytic parameters of different biosensors present in literature is reported. It is evident that a good compromise in terms of applied potential and electrochemical performance was obtained with our nanostructured biosensor.

E. Biosensor performance in cell media

The characterization performed in cell media returned a sensitivity halved with respect to that in PBS (29 and 13.4 µA/(mM cm²), respectively). Note that also the sensitivity obtained with H_2O_2 sensor in cell media is half of that computed in PBS, suggesting that the value of H_2O_2 sensor and glucose biosensor are in good agreement. The concentration range in which the biosensor showed a linear response in cell media was 0.02 – 1 mM. Also the K_m^{app} in cell media (1.6 mM) was half of the values computed in PBS in which the upper limit of linear response is extended to 4.00 mM. Although better performance was obtained in PBS, the values in cell media are encouraging considering the complex matrix of the media in which the calibration of glucose was carried out. The

small value of K_m^{app} suggests that the new Pt nanostructure is a confortable environment for enzyme that maintains its catalytic activity. Moreover, the results confirm that the chosen immobilization procedure with glutaraldehyde preserves the enzyme activity. With our proposed nanostructuration, a good affinity toward the glucose detection is retained, even though the electrochemical performance was lower compared to that obtained in PBS. This is a very important aspect because cell media is a complex matrix that contains different molecules that can adsorb and contaminate the electrode surface making difficult the measurements.
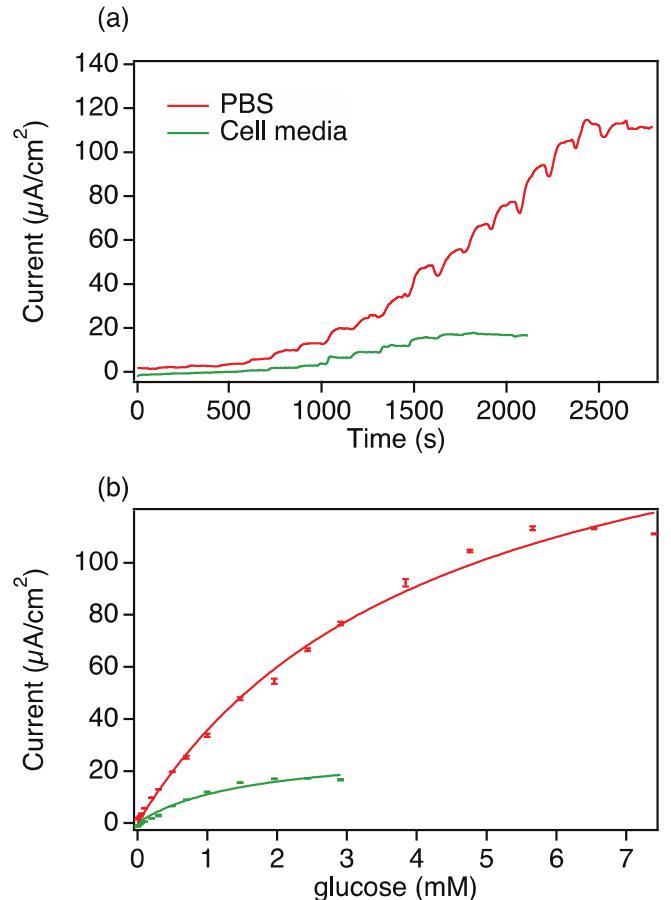

Fig. 3. (a), Chronoamperometry of the biosensor to different concentration of glucose in PBS (red) and in cell media (green); (b) Kinetics of the GOx immobilized on the electrodes in PBS (red) and in cell media (green); applied potential +0.30 V *vs* Ag│AgCl|Cl⁻.

IV. CONCLUSIONS

In this work we have successfully realized a platinum monometallic hybrid nanocomposite by a template-free electrodeposition method including two fast and simple steps. By using two Pt nanostructures, we successfully realized a biosensor with high sensitivity at lower potential than those used in the literature. This result can be attributed to the good catalytic activity of nanoPt and to the combination effect of the two different nanostructures that enhance mass transport at low potential. The results obtained in cell media showed that the realized Pt nanostructure offer a very good microenvironment for enzyme that maintains the catalytic activity and retains an

high affinity towards the glucose. With the first electrochemical experiment we investigate the capability of $Pt_{Ns}Pt_{Nf}$ to oxidize a fixed concentration of H_2O_2 at different potential. The amperometric response increases with the reduction of applied potential from +0.70 V to +0.30 V *vs* Ag│AgCl|Cl⁻ and then it decreases by further lowering the applied voltage. The realized biosensor characterized by chronoamperometry at +0.30 V *vs* Ag│AgCl|Cl⁻ showed good performance. In conclusion, our study provides a simple method to prepare the Pt nanostructured electrodes with a potential application for glucose monitoring in cell cultures.

TABLE I.   COMPARISON OF ELECTROCHEMICAL PERFORMANCE OF SOME GLUCOSE BIOSENSORS

Biosensors	Applied Potential (V)	Linearity range (mM)	Sensitivity ($\mu A/mM\ cm^2$)	Detection Limit (μM)	K_m^{app} (mM)	Ref.
GOD/Pt/CNT/TiO₂	-	0.06 - 1.5	0.24	20000	-	[17]
GC/BNNTs-Pani-PtNPs	+ 0.55 *vs* SCE	0.01 – 5.5	19.02	0.18	3.4	[18]
Pt/MWCNTs/GOx/GC	+ 0.60 *vs* Ag|AgCl	0.001 - 23	58.9	1	14.4	[2]
PMPD-GOD/Pt/nanofibrous PANI	+ 0.60 *vs* SCE	0.002 - 12	-	-	9.34	[19]
GOx/Aunano/Ptnano/CNT/gold	+ 0.60 *vs* SCE	0.5 – 16.5	-	-	10.73	[20]
Nafion/Chit/GOx@PtNCs/Pt	+ 0.60 *vs* Ag|AgCl	0.001 - 8	35.92	0.5	-	[21]
3D porous Pt nanowire array/GOx	+ 0.55 *vs* Ag|AgCl	0.0045 – 189.5	8.74	4.5	14.6	[22]
$Pt_{Ns}Pt_{Nf}$/GOx	+ 0.30 *vs* Ag|AgCl|Cl⁻	0.02 – 4	29 ± 2	6.6 ± 1.5	3.9 ± 0.3	This work

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