A bimetallic nanocoral Au decorated with Pt nanoflowers (bio)sensor for H_2O_2 detection at low potential

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ABSTRACT

In this work, we have developed for the first time a method to make novel gold and platinum hybrid bimetallic nanostructures differing in shape and size. Au-Pt nanostructures were prepared by electrode-position in two simple steps. The first step consists of the electrodeposition of nanocoral Au onto a gold substrate using hydrogen as a dynamic template in an ammonium chloride solution. After that, the Pt nanostructures were deposited onto the nanocoral Au organized in pores. Using Pt (II) and Pt (IV), we realized nanocoral Au decorated with Pt nanospheres and nanocoral Au decorated with Pt nanoflowers, respectively. The bimetallic nanostructures showed better capability to electrochemically oxidize hydrogen peroxide compared with nanocoral Au. Moreover, Au-Pt nanostructures were able to lower the potential of detection and a higher performance was obtained at a low applied potential. Then, glucose oxidase was immobilized onto the bimetallic Au-Pt nanostructure using cross-linking with glutaraldehyde. The biosensor was characterized by chronoamperometry at +0.15 V *vs.* Ag pseudo-reference electrode (PRE) and showed good analytical performances with a linear range from 0.01 to 2.00 mM and a sensitivity of 33.66 $\mu A/mM\ cm^2$. The good value of K_m^{app} (2.28 mM) demonstrates that the hybrid nanostructure is a favorable environment for the enzyme. Moreover, the low working potential can minimize the interference from ascorbic acid and uric acid as well as reducing power consumption to effect sensing. The simple procedure to realize this nanostructure and to immobilize enzymes, as well as the analytical performances of the resulting devices, encourage the use of this technology for the development of biosensors for clinical analysis.

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1. Introduction

Precision medicine relies on the accurate acquisition of large data sets through biosensors as well as data processing including a variety of methods, among which machine learning is of paramount importance. The acquisition of large-scale biomedical data, often performed in real time, poses a strong challenge to the design of the front-end sensing structure and circuitry, because of the requirements of being accurate and achieved within a power-consumption envelope. Within this perspective, the co-design of sensing structures, circuits and data processing means is essential. Here we show how to nanostructure electrodes to achieve biosensing with low power consumption.

Electrochemical biosensors based on the detection of hydrogen peroxide (H_2O_2) were one of the very first ones because H_2O_2 is the one of the main products in many reactions catalysed by oxidases. In the last years the use of nanostructured-based modified electrodes able to drastically lower the applied potential gave new interest on the use of these biodevices [1,2]. In fact, although the first generation biosensors are still widely used, the principal drawback is that H_2O_2 is sensed at a high potential (+0.70 V *vs.* Ag|AgCl) on most electrode materials and possible interfering components present in biological fluids such as ascorbic acid (AA), uric acid (UA) and acetaminophen (APAP) can be oxidized at this high potential and generate a faradic current that interferes in the measurement of the analyte [3,4]. In order to minimize the oxidation of interfering components, it is preferred to work in a potential range from −0.2 to +0.15 V *vs.* Ag|AgCl [5,6]. To overcome the problem of interferences in a real matrix, the research was focused on developing different strategies. The most popular approach reported in

the literature, requires a redox mediator such as Prussian Blue to modify the electrode surface and, in this case, H_2O_2 can be sensed at a low potential (around +0.0 V) avoiding the interference of electroactive molecules [7–9]. A possible alternative is to realize a bienzymatic biosensor with the incorporation of peroxidase, which can communicate through direct electron transfer between the active site of the enzyme and the electrode surface [10–12]. Despite the advantage of these approaches, different disadvantages arise. It is known that many enzymes require neutral or alkaine pH but at this pH value Prussian Blue is unstable and will deteriorate [7]. The main disadvantages of the bienzymatic biosensor are the instability due to the presence of two enzymes and the difficulty in the binding of peroxidases onto solid surfaces [3]. With the advent of nanotechnology, an interest was focused on developing new nanomaterials and to employ them for an improved sensor sensitivity and for a preservation of the native structure of the enzyme when immobilized onto the electrode surface [18–25]. In particular, more recent studies demonstrate that the electrode modification with metal nanoparticles such as gold [4,13,14] or platinum [15–17] decreases the overpotential for the detection of H_2O_2. Nanostructures based on transition metals such as Au and Pt, thanks to their capability to enhance the electrocatalytic activity towards H_2O_2 oxidation, have been incorporated into electrode surfaces to make biosensors [26–30]. Different studies demonstrated that the combination of two metallic nanostructures generates a new structure with different chemical and physical properties that were not observed by using one single metallic nanocatalyst alone [31–33]. According to the literature, AuPt hybrid nanostructures have become more attractive thanks to the synergistic catalytic effect and an excellent resistance to poisoning [34–39]. Different strategies to realize AuPt hybrid structures are reported in literature such as the dealloying method that involves multiple steps [40,41].

In this work we present a simple method for the development of Au-Pt bimetallic nanostructures, by using an electrodeposition method thus obtaining several advantages compared to chemical and physical techniques such as the purity of the new nanostructures and the good control of the dimension of the nanofeatures [42–44]. We demonstrated the synergic effect of Au in combination with Pt to reduce the detection potential of H_2O_2. In particular, we realized, to the best of our knowledge and for the first time, two different AuPt hybrid nanostructures consisting of Au nanocorals decorated with Pt nanoflowers ($Au_{Nc}Pt_{Nf}$) and of Au nanocorals decorated with Pt nanospheres ($Au_{Nc}PtN_{sph}$). The realization of these structures required only two simple electrodeposition steps. The resulting structures were firstly characterized by scanning electron microscopy (SEM). A chronoamperometric study of H_2O_2 detection using bare Au and Pt, Au and Pt nanostructures and AuPt hybrid nanocomposites was performed at different potentials. $Au_{Nc}Pt_{Nf}$ exhibited the best performance for H_2O_2 detection. Successively, glucose oxidase (GOx) was utilized as a model oxidase and a first generation glucose biosensor was fabricated immobilizing the enzyme by cross-linking with glutaraldehyde [45] onto an $Au_{Nc}Pt_{Nf}$ modified electrode. The realized glucose biosensor was characterized by chronoamperometry at +0.15 V vs. Ag PRE and the effect of interfering species (AA, UA, APAP) was also evaluated.

2. Results and discussion

2.1. Morphological characterization

In Fig. 1 SEM images of the various Au-Pt hybrid nanostructures are shown. With the first electrodeposition step, a layer of Au nanocorals was formed, which is organized in pores (Figs. 1(a and b)). The morphological characterization of the Au nanocorals

was described in our previous work [46]. Au nanocorals present different sites for the attachment of either Pt nanoflowers or Pt nanospheres realized in the second electrodeposition step. In our previous work [47], we demonstrated that the composition of the solution and the applied potential during the electrodeposition are important parameters to obtain Pt nanostructures differing in shape and size. In the magnification of Fig. 1a, Au nanocorals obtained from $HAuCl_4$-containing solutions is shown. Fig. 1b shows Pt nanoflowers obtained with H_2PtCl_6 and uniformly electrodeposited outside of the Au pores. Similarly, also Pt nanospheres obtained with K_2PtCl_4 were only formed outside of the Au pores as shown in the magnification of Fig. 1c.

2.2. Electrooxidation of H_2O_2

Glucose can be indirectly determined by sensing H_2O_2 product of the following enzymatic reaction [8]

$$\beta\text{-}D\text{-glucose} + O_2 + H_2O \leftrightarrow D\text{-gluconic acid} + H_2O_2 \tag{1}$$
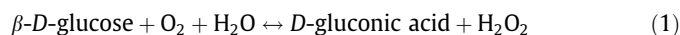
To evaluate the catalytic activity toward H_2O_2 oxidation, a series of measurements were carried out using the different modified electrodes described above. In the first experiment, the amperometric response to 20 µM H_2O_2 was evaluated as a function of the applied potential using different surfaces. The potential was varied from +0.7 to +0.2 V vs. Ag PRE and the results are shown in Fig. 2. It can be seen that for a bare Au electrode, the current response increases with an increase in the applied potential from +0.3 to +0.7 V and no response was obtained at a potential lower than +0.3 V. With Au nanocorals, the amperometric response was observed also at +0.2 V and the current increased with an increase in potential from +0.2 to +0.7 V. Interestingly, with both bimetallic nanostructures, not only the current reaches a higher value but the amperometric response, obtained in 100 s, increases by decreasing the applied potential from +0.7 to +0.2 V. These results suggest that the capability to improve the amperometric performance with a reduction in the applied potential, could be due not only to the excellent catalytic properties of Pt and the realized nanostructures but also to the synergic effect due to the combination of Au and Pt nanostructures. Moreover, if comparing the results of the two different bimetallic nanostructures ($Au_{Nc}Pt_{Nf}$ and $Au_{Nc}PtN_{sph}$), it should be noted that although the trend is the same, Pt nanoflowers have a shape that give a major contribute to enhance the catalytic performance toward H_2O_2 detection compared to Pt nanospheres. The explanation might be that Pt nanoflowers exhibit a more nanostructured surface than Pt nanospheres. The higher current acquired with $Au_{Nc}Pt_{Nf}$ than $Au_{Nc}PtN_{sph}$ is due to a major surface area to electrooxidize H_2O_2. Since our goal is to realize a biosensor with high performance, we have chosen to work with $Au_{Nc}Pt_{Nf}$ in the successive study.

2.3. Effect of the potential on the sensitivity

The response to different concentrations of H_2O_2 was investigated in order to calculate the sensitivity for H_2O_2 at different applied potentials using the three different steps involved in making the $Au_{Nc}Pt_{Nf}$. The calibration experiments were carried out in the concentration range between 20 µM and 100 µM at different potentials using Au bare, Au nanocorals and $Au_{Nc}Pt_{Nf}$; the response currents obtained are shown in Fig. 3(a–c). The trends confirm the data obtained in the previous experiments. With Au bare, the sensitivity increased with an increase in applied potential. With Au nanocorals the sensitivity is higher than that one obtained with Au bare and the sensitivity increased with an increase in applied potential. As expected, the best results have been achieved with the bimetallic nanostructures, where the sensitivity increased with a decrease in the applied potential, suggesting that the combina-