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Bubble electrodeposition of gold porous nanocorals for the enzymatic and non-enzymatic detection of glucose

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ABSTRACT

Au nanocorals are grown on gold screen-printed electrodes (SPEs) by using a novel and simple one-step electrodeposition process. Scanning electron microscopy was used for the morphological characterization. The devices were assembled on a three-electrode SPE system, which is flexible and mass producible. The electroactive surface area, determined by cyclic voltammetry in sulphuric acid, was found to be 0.07 ± 0.01 cm^2 and 35.3 ± 2.7 cm^2 for bare Au and nanocoral Au, respectively. The nanocoral modified SPEs were used to develop an enzymatic glucose biosensor based on H_2O_2 detection. Au nanocoral electrodes showed a higher sensitivity of 48.3 ± 0.9 μA/(mM cm^2) at +0.45 V *vs* Ag|AgCl compared to a value of 24.6 ± 1.3 μA/(mM cm^2) at +0.70 V *vs* Ag|AgCl obtained with bare Au electrodes. However, the modified electrodes have indeed proven to be extremely powerful for the direct detection of glucose with a non-enzymatic approach. The results confirmed a clear peak observed by using nanocoral Au electrode even in the presence of chloride ions at physiological concentration. Amperometric study carried out at +0.15 V *vs* Ag|AgCl in the presence of 0.12 M NaCl showed a linear range for glucose between 0.1 and 13 mM.

1. Introduction

In recent years, the research in the field of electrochemical sensors constantly increased thanks to features such as miniaturization, low cost, simplicity in use, and fast response. Unfortunately, bare electrodes often suffer from disadvantages such as low sensitivity and show interferences by electroactive substances always present in real matrices that limit their usage especially in clinical and food analyses [1–4]. One of the strategies followed has been to increase the performances of these electrochemical sensors through modification of the electrode surface, thus enhancing the electrochemically active surface area and the mass transport effect. Among the different approaches found in literature to increase the electrode surface area, the most effective employ electrode modifications by means of nanomaterials. In this field of research, several nanostructures have been used, including but not limited to carbon [5] or titanium [6] nanotubes, gold [7] or semi-conducting [8] nanoparticles, graphite [9] or graphene nanoflowers [10], fullerenols [11], conductive polymers [12], and nanoporous gold [13]. To this aim, we have evaluated the use of nanocoral gold, which displays several advantages including a greater surface area compared to bare electrodes, better electron transport and, thanks to the higher surface area, are able to host a greater amount of enzyme when used as an electrochemical biosensor [14,15]. The common techniques to realize these structures are all attributable to two main approaches, namely: the dealloying and templating methods [16,17]. In this work, we report the realization of porous gold incorporating nanocorals by using hydrogen bubbles as a dynamic template. The structures present a highly rough surface and are obtained by means of a technique recently reported in the literature that consists of a simple one step electrodeposition at high overpotential without the need of any successive removal of the template [18]. It is very important to obtain devices with high performances as well as to fabricate them with a method suitable for mass production, free of contaminants, fast, cheap, and easy to prepare. Screen-printed electrodes (SPEs) have been used especially in the development of miniaturized biosensors thanks to their many advantages such as flexibility of design and good reproducibility. They are also very much used in mass production of biosensors due to their low fabrication cost [19,20]. Here we report the optimization of a porous structure based on nanocorals to modify the gold working surface of SPEs. The realized structures have been characterized by scanning electron microscopy (SEM) and electrochemical analysis. In order to assess the real

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benefits of a nanocoral structure for electrochemical sensing of analytes, the obtained modified electrode was tested with ferricyanide. Moreover, we also realized a first-generation glucose biosensor by immobilizing glucose oxidase (GOx) on the porous electrode surface by using glutaraldehyde as an immobilizing reagent. It is known in literature that the detection of H_2O_2 by using a gold bare electrode suffers from large overvoltages (+700 mV *vs* Ag|AgCl) and other interfering species present in biological fluids such as ascorbic acid (AA) and uric acid (UA) could be oxidized at this high potential and generate a faradic current that interferes with the measurement of the analyte [21–23]. The performance of the biosensor modified with nanocorals Au was tested by measuring the H_2O_2 produced from the GOx reaction at +450 mV *vs* Ag|AgCl. Another application of these nanostructures is the non-enzymatic detection of glucose that got recently an increased interest in the literature [17,24,25]. Compared with enzymatic detection, a non-enzymatic analysis presents more stability, reproducibility and also is oxygen limitation-free [26]. The electrochemical oxidation of glucose at gold electrodes occurs at more negative potentials than on other noble metals and carbon electrodes. Also for the oxidation of glucose research in the field of nanostructures is increased thanks to the different advantages of nanostructured electrodes compared to their bare counterparts. Moreover, the nanostructuring allows the electrode to become resistant to fouling and interfering components [26,27]. Therefore, the capability of such coral-like nanostructures to oxidize glucose under physiological conditions (in particular in the presence of normal chloride ion concentrations) was also carefully studied.

2. Experimental procedure

2.1. Chemicals

Gold (III) chloride hydrate from Sigma and ammonium chloride from BioChemica were utilized for making macroporous gold. H_2SO_4 (95–98%), potassium chloride (minimum 99.0%), ascorbic acid, uric acid, potassium hexacyanoferrate (III) (99%) and D-(+)-glucose were purchased from Sigma. Glucose oxidase (GOx) grade I, 2 MU/5.06 g was obtained from Roche Diagnostics. Glutaraldehyde solution, grade II, 25% from Sigma was utilized to immobilize GOx. Phosphate buffer saline from Sigma at pH 7.4 if not differently specified was used to prepare the solutions.

2.2. Synthesis of nanocoral gold

Before the electrodeposition, the surface of solid gold electrodes was cleaned by cyclic voltammetry in the range of −0.2 to 1.2 V in a solution of 0.1 M H_2SO_4 at the scan rate of 100 mV s^{-1} until reproducible voltammograms were obtained [28,29]. Nanocoral gold was directly deposited onto a solid gold substrate by electrodeposition using the hydrogen dynamic template. We used the gold of a SPE as the working and the carbon as the counter and silver as a reference of another SPE and the two SPEs were immersed in a solution of 0.01 M $HAuCl_4$ and 2.5 M NH_4Cl [18]. Gold was then electrodeposited by applying a fixed potential of −3.0 V under stirring conditions for 15, 60 and 120 s.

2.3. Biosensor assembly

In case of enzymatic biosensors, GOx was immobilized onto bare Au and nanocoral modified Au electrodes by cross-linking using glutaraldehyde [30]. Glutaraldehyde 2.5% (50 μl) was mixed with 10 mM PBS solution (950 μl) containing 15 mg of GOx (5930 U). Then, the enzyme-based solution was homogenized by vortex mixing and 10 μl of this solution was spread on the working electrode. The biosensors were kept overnight at 4 °C before the measurements.

2.4. Characterization of nanocoral gold by SEM

A Zeiss Merlin Scanning Electron Microscope (SEM) was used to investigate the morphological change of the gold electrode after the electrodeposition (voltage 5 kV).

2.5. Electrochemical measurements

For cyclic voltammetry (CV) and chronoamperometric experiments a potentiostat (Metrohm, Autolab PGSTAT101) was used with the NOVA software (Eco Chemie, The Netherlands) with a conventional three-electrode configuration. SPEs (Metrohm) with a gold working electrode (4 mm diameter), carbon as counter electrode and silver reference electrode were used. The electroactive surface area, the measurements of potassium hexacyanoferrate, the direct oxidation of glucose were estimated with CV. Chronoamperometry under stirring conditions was performed to determine the electrochemical performance of the glucose biosensor. The sensitivity values were calculated per projected cm^2. IgorPro software was used for the data elaboration. All potentials are referred to Ag|AgCl reference electrode. All experiments, including electrodepositions, were carried out under aerobic conditions, at room temperature.

3. Results and discussion

3.1. SEM images

In the dynamic bubble template electrodeposition, hydrogen evolution is very important and only a high concentration of NH_4^+ increased the rate of evolution of hydrogen [31]. We have found that the gold nanocorals were organized in the pores on the gold SPEs at a high overpotential of −3.0 V *vs* Ag|AgCl. The composition of the solution is important because only in the presence of 2.5 M NH_4Cl a sufficient amount of bubbles is generated to realize the foam resulting in a porous structure. The concentration of $HAuCl_4$ also plays a crucial role in the formation of an ordered honeycomb structure because no pores but dendritic structures are observed with a higher amount of gold in solution. During electrodeposition, gold is deposited in the interstitial spaces of the bubbles, and successively the bubbles detach from the surface of the electrode leaving a porous material formed on the surface [18,32]. Then the bubble template is removed during the same step of deposition of gold and the resulting material was free from impurity. Du Toit and Di Lorenzo [14] utilized a similar technique to realize a porous structure for sensing applications. However, their procedure consists of two steps. Conversely we utilized only one step in this work. We noted that also in the presence of only 0.010 M of $HAuCl_4$ the honeycomb structure is formed. This value of concentration is one order of magnitude lower than that reported in the literature for the realization of gold nanofoam and that means less costly. In Fig. 1a–c, SEM images at low and high magnification of Au deposited at −3.0 V for 15, 60 and 120 s are exhibited. As shown, the deposited gold has a coral-like structure in nm size. After 15 s (see Fig. 1a), gold begins to be deposited onto the solid gold substrate in the form of coral-like structures but no pores appear. Prolonging the time of deposition until 60 s, the nanocoral continues to grow (Fig. 1b) and the first pores are formed. After 120 s, a very evident honeycomb structure is formed with porous in the μm size (Fig. 1c). This structure presents a highly rough surface thanks to the nanocorals that are forming the porous film and therefore this structure was used for further experiments.

3.2. Electrochemical characterization of nanocoral Au SPEs

In Fig. 2a, CVs in a 0.1 M H_2SO_4 solution at 100 mV s^{-1} of nanocoral Au prepared using the hydrogen dynamic template are shown. The electrochemical behaviour of the electrodeposited material changes as the deposition time increases: in particular, the peak at +0.60 V increases