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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

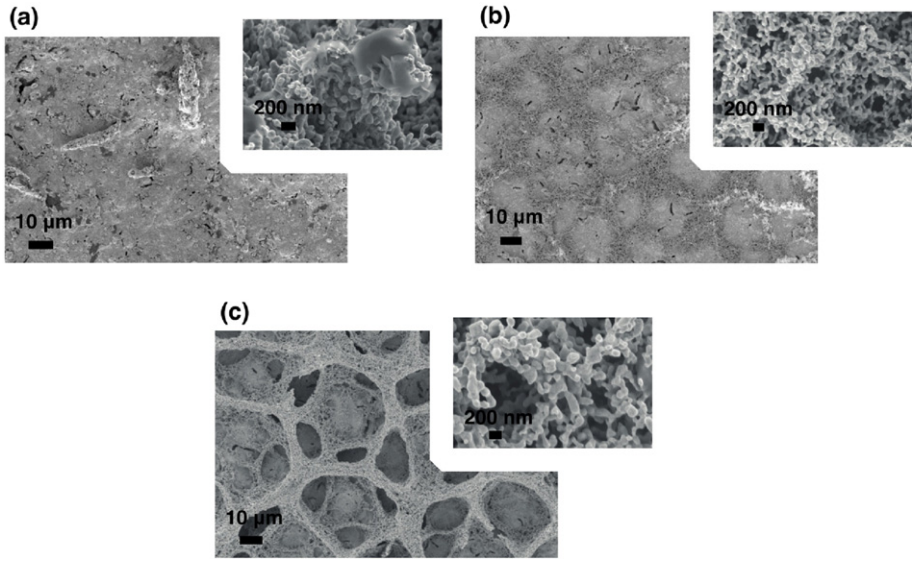

Fig. 1. SEM images of porous gold nanocoral-like structures deposited at −3.0 V for 15 s (a), 60 s (b) and 120 s (c) in a solution of 0.01 M $HAuCl_4$ and 2.5 M NH_4Cl. Insets show the respective magnifications.

with the time of electrodeposition. This peak is due to the electrochemical reduction of gold oxide formed during the anodic scan and it is proportional to the active surface area [33]. The increase in this peak is a proof that the amount of gold deposited from 15 to 120 s is increasing the electrochemical surface area [34–35].

The integral of the peak related to the Au oxide reduction in CV and acquired in 0.1 M H_2SO_4 (Autolab, Metrohm, Switzerland) at a scan rate of 100 mV/s was calculated using the Nova software (Autolab, Metrohm) after the subtraction of the electrochemical double layer current [36].The related charge value (Q) was evaluated by dividing the integral (A V) by the scan rate (V/s). The value of electroactive area was calculated as Q (μC)/386 μC/cm^2 where 386 μC/cm^2 is related to the theoretical estimation for an atomically 1 cm^2 smooth Au electrode [36]. According to this method, the electroactive surface areas were calculated to be 0.07 ± 0.01 cm^2 and 35.3 ± 2.7 cm^2 for the bare Au and nanocoral Au electrode, respectively. The roughness factor is very important for the activity of the gold electrode, because the increase in surface roughness results in an increase in electrochemical activity [27]. It is known that the roughness has an influence on kinetically controlled sluggish reactions, such as the oxidation of glucose for enhancing the faradic current. In contrast, the oxidation of ascorbic acid is a diffusion-controlled fast reaction, which is independent on the roughness of the electrode surface [24]. It is evident that the roughness represents a very important aspect for the oxidation of glucose in biological fluids due to typical interfering components, such as ascorbic acid and uric acid. The roughness factor was calculated as the ratio between the real surface area electrochemically measured and the geometrical area of electrode [33] and it was found to be equal to 0.58 ± 0.07 and 281.1 ± 21.4 for the bare Au and the nanocoral Au, respectively.

Moreover, the presence of a nanocoral-like structure increases the faradic current towards the sensing of potassium hexacyanoferrate and was an effect of an increased real surface area (see Table 1). The peak-to-peak separation (ΔE_p) in potassium hexacyanoferrate-based solutions (20 mM) was calculated by carrying out CVs at 100 mV s^{-1} in 0.1 M PBS solution (pH 6.6). Table 1 shows the decrease of ΔE_p when nanocoral Au is used rather than bare Au. The lower value of ΔE_p for nanocoral Au indicates a more reversible and a faster electron transfer reaction than using the bare electrode. Moreover, the value of half-wave potential $E_{1/2}$ obtained with bare Au and nanocoral electrode is almost constant, thus indicating that the modified electrode retains the reversibility of the voltammetric signal.

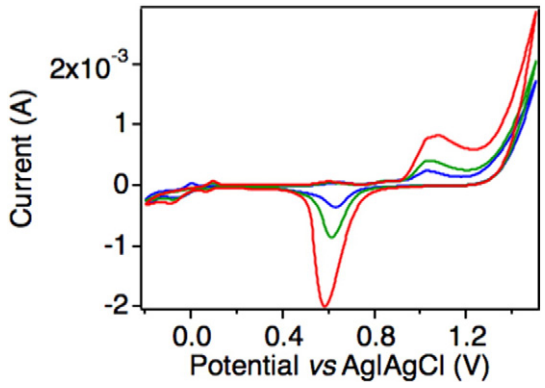

Fig. 2. (a) CVs registered in 0.1 M H_2SO_4 at 100 mV/s for Au deposited at −3.0 V for 15 s (blue), 60 s (green) and 120 s (red) at a scan rate of 100 mV s^{-1}.

3.3. Enzymatic detection of glucose with a biosensor based on GOx modified nanocoral Au

Glucose oxidase was chosen as a model oxidase enzyme to evaluate the feasibility of the proposed material as an electrochemical transducer, since glucose is typically indirectly determined by measuring the hydrogen peroxide produced according to the following reaction [37]:

$$\text{Glucose} + O_2 + H_2O \overset{GOx}{\rightleftarrows} \text{Gluconic acid} + H_2O_2. \quad (2)$$

The enzymatic solution with glutaraldehyde was prepared as described in Section 2.3. A total of 5930 U of enzyme was deposited onto Au bare and Au modified electrodes.

Table 1
Anodic (I_{pa}) and cathodic (I_{pc}) peak currents, ΔE_p and $E_{1/2}$ obtained from CVs in potassium hexacyanoferrate-based solutions (20 mM) in 0.1 M PBS pH 6.6 (scan rate: 100 mV s^{-1}).

$K_3[Fe(CN)_6]$ 20 mM	I_{pa} (μA)	I_{pc} (μA)	ΔE_p (mV)	$E_{1/2}$ (mV)
Au bare	384.0 ± 1.7	−387.8 ± 3.8	85.5 ± 1.0	270.4 ± 0.5
Au nanocorals	800.6 ± 30.7	−798.4 ± 27.2	48.2 ± 2.5	274.4 ± 0.6

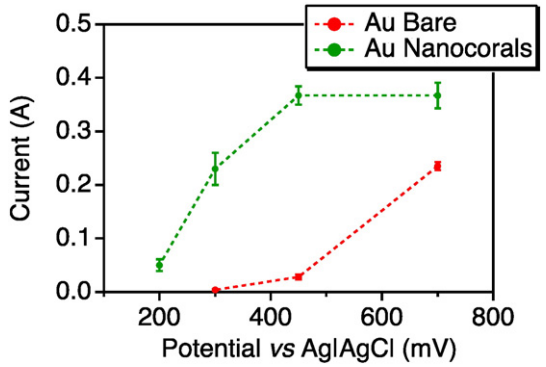

Fig. 3. Effect of the applied potential on the response to 20 μM H_2O_2 obtained for Au bare (red) and Au nanocoral (green). Experiment carried out in 0.01 M PBS solution pH 7.4.

In this experiment, the amperometric response to 20 μM H_2O_2 was evaluated as a function of the applied potential using Au bare and Au modified electrodes. The potential was varied from +0.7 to +0.3 V *vs* Ag|AgCl and the results are shown in Fig. 3.

It can be seen that with bare Au the highest current response is obtained at +0.70 V while with nanocoral Au the amperometric response remains almost constant in the range + 0.70 to +0.45 V and decreases by decreasing applied potential until +0.30 V. It is important to note that the nanostructuration not only increases the electrochemical surface area but also lowers the detection potential for H_2O_2. This result means a reduction of the interfering species. Then the potential of +0.45 V was chosen for the characterization of GOx/Au nanocoral biosensor, while Au bare biosensor was characterized at a potential of +0.70 V.

Fig. 4 shows plots of current response obtained after successive addition of glucose as a function of time using bare Au and nanocoral Au electrodes as GOx biosensor transducers. It should be noted that Fig. 4 did not compare the amperometric response of the two biosensors at the same fixed potential. The response of these biosensors was obtained on the successive addition of glucose at a working potential of +0.70 V and +0.45 V *vs* Ag|AgCl for bare Au and nanocoral Au, respectively, under stirred conditions in 0.01 M PBS. With this experiment we demonstrated that it is possible to work at a low potential by using Au nanocoral towards the hydrogen peroxide electrooxidation as a product of GOx reaction. The current response increases by increasing glucose concentrations for both biosensors, but the nanocoral Au based biosensor exhibited a higher current and, consequently, better sensitivity compared to the bare Au based biosensor. The apparent Michaelis–Menten constant K_m^{app}, which refers to heterogeneous catalysis depends both on the interaction between the enzyme and the electrode and on the amount of immobilized enzyme. This value was calculated by using the Lineweaver–Burk method. Values of 1.5 ± 0.3 mM and 3.2 ± 0.3 mM were estimated for GOx immobilized onto bare Au and nanocoral Au electrodes, respectively. As observed in Table 2, we obtained higher value of I_{max} measured with the GOx/nanocoral Au at 450 mV compared to that obtained at 700 mV with bare Au; the K_m^{app} value calculated in the case of the nanocoral Au based biosensor increases as well as the linearity range. These findings are in good agreement with those reported in the literature by Szamocki et al. [30], Gamero et al. [38] and Gorton et al. [39].

Also the sensitivity resulted in higher values (24.6 ± 1.3 and 48.3 ± 0.9 μA/(mM cm^2) with the bare Au and nanocoral Au, respectively) indicating that the presence of the nanocorals in the porous structures makes glucose more accessible to the active site of the enzyme, thus improving the analytical performances. The value of enzymatic efficiency reported in Table 2 as I_{max}/K_m^{app}, increased with the nanostructured biosensors indicating that the enzyme maintained its native structure. The wide linear range and higher sensitivity can be attributed to the excellent electrocatalytic activity of the nanocoral Au/porous structure as a catalyst. It should be noted that +0.45 V was selected as a working potential that is substantially lower than the potential commonly utilized for the first generation biosensors based on electrochemical oxidation of the H_2O_2 produced, see reaction (2).

Table 3 gives values that show the analytical performance of different GOx biosensors as comparison. We compare in this table that only GOx biosensors modified with nanostructures of Au. It should be noted that the biosensor fabrications found in other works consist complex procedures of nanostructurations such as the combination of nano-Au and other metals [14,40–42] or carbon nanotubes by employing multiple steps [41]. Although the applied potential is the same, we obtained a better sensitivity with our sensor as compared to the work in [43]. It can be seen that the nanocoral Au modified biosensor showed the best compromise between high sensitivity and low potential applied.

3.4. Non-enzymatic detection of glucose with nanocoral Au SPEs

Generally a bare electrode is not an ideal substrate for the oxidation of glucose because the reaction is kinetically restricted and the sensitivity decreases with time due to the fouling of the electrode surface caused by both side reaction products and other molecules usually present in real samples. For example glucose can in principle be directly oxidized at Au electrodes but a high amount of chloride ions are adsorbed onto the surface in neutral conditions [26,27]. Solid Au electrodes can be routinely used for the determination of glucose and other carbohydrates at high pH using the pulsed amperometric technique, usually used in combination with liquid chromatography [43]. However, the requirements of using a very corrosive mobile phase of high pH in combination with a complex electrochemical detection technique that requires a more advanced potentiostat make this technique less attractive for use in less specialized laboratories.

Nanostructures are known as good catalysts in non-enzymatic detection without showing typical signals of the interfering compounds

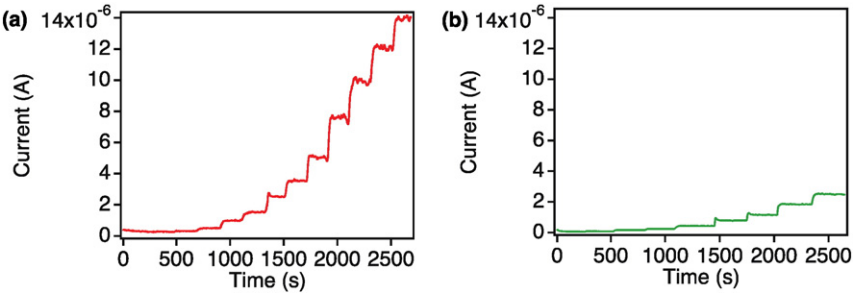

Fig. 4. Amperometric response obtained to successive additions of glucose in a stirred 10 ml solution of PBS 0.01 M pH 7.4 at +0.70 V and +0.45 V (*vs* Ag|AgCl) using nanocoral Au (a) and bare Au (b) biosensors, respectively.

Table 2
Kinetic and electroanalytic parameters obtained with GOx biosensors employing bare Au and nanocoral Au obtained by amperometry at a fixed potential of +700 mV and +450 mV respectively using glucose as substrate.

	K_m^{app} (mM)	I_{max} (μA)	I_{max}/K_m^{app} (μA/mM)	Linearity range (mM)	Sensitivity (μA/mM cm^2)	r^2
GOx/Au bare	1.5 ± 0.3	8.3 ± 0.6	5.4 ± 1.3	0.01–1.00	24.6 ± 1.3	0.99
GOx/Au nanocorals	3.2 ± 0.3	34.0 ± 1.3	10.7 ± 1.7	0.005–3.00	48.3 ± 0.9	0.99

Table 3
Comparison of electrochemical performances of glucose biosensors.

GOx biosensors	Applied potential	Linear range (mM)	Sensitivity	K_m (mM)	Ref.
GOx/Au_{nano}/Pt_{nano}/CNT/gold	+0.60 V	0.5–16.5	–	10.73	[40]
GOD/GNp/CS/GOD/GNp/PAA/Pt	+0.60 V	0.5–16	0.555 μA mM^{-1}	10.5	[41]
GOD/ConA/Nano-Au/Pt-Au@ZnONRs/GCE	+0.45 V	1.8×10^{-3} – 5.15	3.26 μA mM^{-1}	0.41	[42]
GOx-hPG	+0.52 V	5×10^{-3} – 10	0.71 μA mM^{-1}	6.3	[14]
Nanocoral Au	+0.45 V	0.005–3.00	6.07 μA mM^{-1}	3.2	This work

[17]. In fact, the limitation to utilize a sensor for the detection of glucose in biological fluids is that chloride ions inhibit the oxidation of glucose when a bare substrate is utilized, while a porous nanostructure is able to avoid the interference during the electro-oxidation [24]. The higher electroactive surface area of the nanomaterials enhances the electro-oxidation and is ideal for glucose oxidation under kinetic control [27]. To evaluate the possible future application of nanocoral gold electrode for the direct determination of glucose in a serum sample, we performed preliminary experiments under physiological concentration of chloride ions that are the main inhibitors of the direct glucose oxidation. CVs in a solution of 20 mM glucose in the absence and presence of 0.12 M NaCl were performed. As shown in Fig. 5a, the CVs obtained in the absence and presence of NaCl at physiological concentration utilizing a bare Au electrode are equivalent, indicating that a bare structure presented no catalytic activity towards glucose oxidation. Conversely, the nanocoral Au shown in Fig. 5b a strong catalytic activity for glucose oxidation in the absence of chloride ions (blue curve) at +0.15 V (*vs* Ag|AgCl). The peak is due to the reaction of the hydrogen atom at carbon C1 of the aldehyde group of glucose. The obtained CVs are similar to those reported in the literature by Du Toit and Di Lorenzo [44]. The oxidation peak decreases in the presence of 0.12 M NaCl but it is still evident, suggesting that a porous structure is a promising substrate for the electrochemical oxidation of glucose in a non-enzymatic electrochemical analytical method. In order to verify that a high roughness surface does not favour the diffusion controlled reaction, CVs were registered in solutions containing physiological concentrations of ascorbic acid (AA) and uric acid (UA). As known, AA and UA are oxidized at positive potentials and the generated current can interfere with the response signal for glucose [4]. In Fig. 5c, CVs in PBS and in a solution of physiological concentrations of AA and UA using the nanocoral Au based sensor are shown. As shown in Fig. 5c, no peak was observed around 0.15 V in the presence of AA and UA at physiological concentrations as the

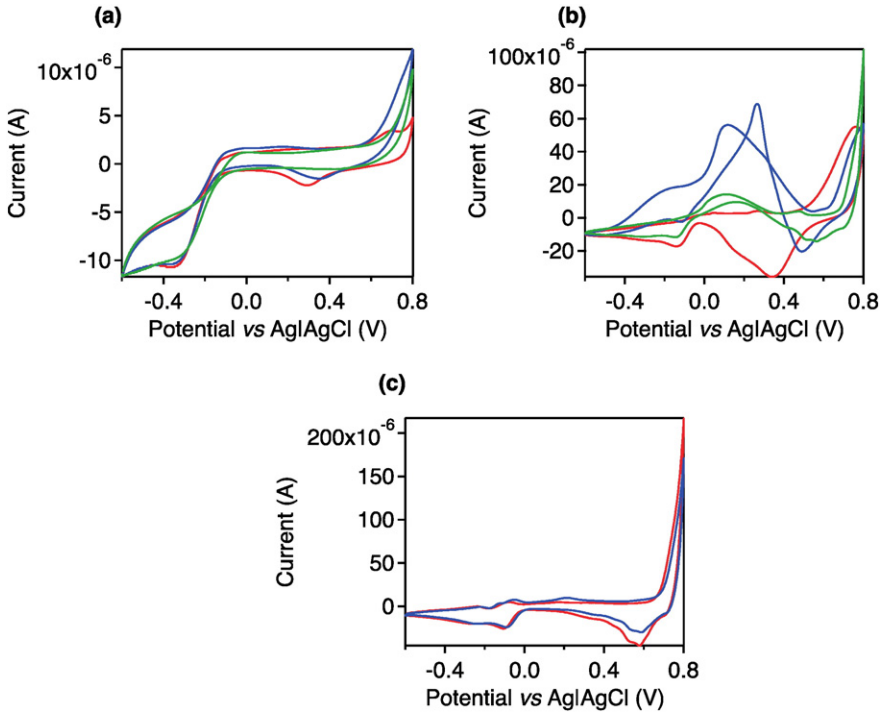

Fig. 5. CVs in blank solution (red), 20 mM glucose in the absence of 0.12 M NaCl (blue) and 20 mM glucose in the presence of 0.12 M NaCl (green) using bare Au (a) and nanocoral Au electrode (b) in 0.01 M PBS pH 7.4. In (c) CVs in blank solution (red), and 0.1 M AA and 0.01 M UA (blue) using nanocoral Au electrodes are shown.

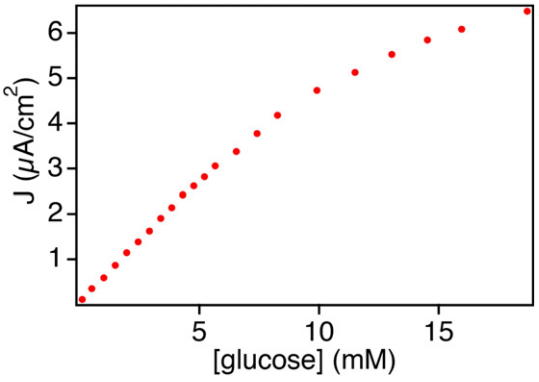

Fig. 6. Current density as a function of concentration of glucose. The amperometric response was obtained to successive addition of glucose in a stirred 10 ml PBS pH 7.4 containing a physiological concentration of NaCl (0.12 M) at +0.15 V (*vs* Ag|AgCl) using a nanocoral Au based electrode.

porous structure is not a good substrate for the oxidation of these compounds. In fact, a porous electrode with a high value of roughness factor is a good substrate for the direct detection of glucose that is a kinetically controlled sluggish reaction. On the other hand, AA and UA electrooxidations are diffusion controlled and for this reason they do not depend on the roughness [17,24].

By considering the CVs exhibited in Fig. 5a–c, a potential of +0.15 V was chosen to perform the amperometric detection of glucose in phosphate buffer in the presence of a physiological concentration of chloride ions. The plot of the current as a function of concentration of glucose is shown in Fig. 6.

The current increased by increasing the concentration of glucose and showed a linear response in the range of 0.1 to 14 mM with a sensitivity of 0.5 μA/mM cm^2. Then, glucose is normally present in biological fluids in the range 3–8 mM and can thus be determined in the presence of a possible interfering compound, the concentration levels of which are almost 30 times higher than those of glucose [17]. In Table 4 the analytical performance of different non-enzymatic glucose sensors are reported. It can be seen that the nanocoral Au based sensor showed a high sensitivity at a low potential and a wide linear response range.

4. Conclusions

Nanocorals in porous structures have been synthesized onto gold SPEs by using hydrogen bubbles as the dynamic template. In the present work we demonstrated that the modified electrodes showed an enhancement of the biosensor performance. By using ferricyanide as an electrochemical probe, we recognize that the modified surface improves the electron transfer and the reversibility as seen from the higher current peak and the low peak-to-peak separation than a corresponding bare electrode. Considering the low standard errors of the measurements of electroactive surface area, characterization in ferricyanide and experiments in H_2O_2-containing solutions, we could conclude that the proposed electrodes present a good reproducibility.

The nanocoral Au modified electrodes were used for enzymatic and non-enzymatic detection of glucose. In the first case a GOx based biosensor has been realized based on H_2O_2 detection at a very low potential (+0.45 V) and with high sensitivity. In the second case we demonstrated that the increase in roughness facilitates the direct electrochemical oxidation of glucose even in the presence of a physiological concentration of chloride ion, a well-known inhibitor of this type of oxidation. It has also been demonstrated that it is possible to determine glucose in the presence of interfering compounds such as ascorbic acid and uric acid.

In conclusion, nanocoral Au in porous electrodes are promising structures for sensing and biosensing applications thanks to the simplicity in the fabrication procedure and to its extremely good electrocatalytic performances.

Table 4
Different porous Au based non-enzymatic glucose sensors.

Electrode surface	Potential	Linear range (mM)	Sensitivity (μA/mM cm^2)	Ref.
Porous Au	+0.31 V	2–10	11.8	[17]
Porous Au	+0.35 V	3×10^{-3}–7.7	Not given	[45]
3D gold film electrode	−0.30 V	5×10^{-3}–10	0.0466	[46]
Porous Au	+0.25 V	2.0–20	0.032	[47]
Nanocoral Au	+0.15 V	0.1–14	0.5	This work

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