Pulse Laser Deposition Fabricating Gold Nanoclusters on a Glassy Carbon Surface for Nonenzymatic Glucose Sensing

Honghui SHU, Gang CHANG,† Zhiqiang WANG, Pai LI, Yuting ZHANG, and Yunbin HE†

Hubei Collaborative Innovation Center for Advanced Organic Chemical Materials, Ministry-of-Education Key Laboratory for the Green Preparation and Application of Functional Materials, Faculty of Materials Science and Engineering, Hubei University, No. 368 Youyi Avenue, Wuchang, Wuhan 430062, P. R. China

A One-step technique for depositing gold nanoclusters (GNCs) onto the surface of a glassy carbon (GC) plate was developed by using pulse laser deposition (PLD) with appropriate process parameters. The method is simple and clean without using any templates, surfactants, or stabilizers. The experimental factors (pulse laser number and the pressure of inert gas (Ar)) that affect the morphology and structure of GNCs, and thus affect the electrocatalytic oxidation performance towards glucose were systematically investigated by means of transmission electron microscopy (TEM) and electrochemical methods (cyclic voltammograms (CV) and chronoamperometry methods). The GC electrode modified by GNCs exhibited a rapid response time (about 2 s), a broad linear range (0.1 to 20 mM), and good stability. The sensitivity was estimated to be 31.18 μA cm⁻² mM⁻¹ (vs. geometric area), which is higher than that of the Au bulk electrode. It has a good resistance to the common interfering species, such as ascorbic acid (AA), uric acid (UA) and 4-acetaminophen (AP). Therefore, this work has demonstrated a simple and effective sensing platform for the nonenzymatic detection of glucose, and can be used as a new material for a novel non-enzymatic glucose sensor.

Keywords Pulse laser deposition (PLD), gold nanoclusters, glucose oxidation, nonenzymatic sensor

(Received March 4, 2015; Accepted May 12, 2015; Published July 10, 2015)

Introduction

The determination of glucose has vital significance in the pharmaceutical industry, food industry, environmental monitoring, and biological fuel cells. In particular, the level of glucose in the blood provides important indicators to measure the metabolism ability and clinical diagnosis and treatment of diabetes. Therefore, much effort has been focused on developing suitable techniques for precisely sensing glucose with high sensitivity, selectivity, reliability, and low cost.¹⁻⁵ Most studies on such subjects are based on glucose oxidase (GOx) bound to electrode transducers, in which hydrogen peroxide is produced from the oxidation of glucose, and could be amperometrically detected to stoichiometric related to glucose levels. Although enzymatic detection shows good selectivity and high sensitivity in the detection of glucose, the activity of enzyme is easy to be affected by the pH, temperature, and toxic chemicals.⁶ The tedious immobilization process of enzyme could be another barrier that limits their further applications.⁷

In order to solve these problems, many attempts have been made to detect glucose based on nonenzymatic electrochemical oxidation, particularly when using nonenzymatic amperometric glucose sensors. The performance of non-enzymatic glucose biosensors greatly depended on the catalytic activity of catalyst materials towards glucose oxidation.⁸⁻¹⁴ Therefore, it not only greatly simplifies structure of the sensor, but also solves the instability of enzyme in conventional biosensors, improving long-term stability. A variety of metals and conventional bulk composite metal material were applied to study nonenzymatic sensors, but the expensive price and low selectivity from any bulk noble metal restricted the development of the nonenzymatic sensor. Considering the high specific surface area, excellent conductivity and catalytic activity, metal nanomaterials have become the research focus of electrocatalysts for glucose sensing. Nowadays, various metal nanomaterials, like Pt, Pd, Au, etc.²⁻¹³ have been studied for the electrocatalytic oxidation of glucose. For Cu electrodes, an alkaline solution environment is frequently required in sensing glucose.⁵ Pt nanomaterial is of great promise because of its extraordinary performance in the electro-oxidation of glucose in neutral buffers, but it is easily poisoned by intermediates and products generated in the experimental processes.¹⁴ Gold is an attractive metal for the glucose electrooxidation reaction, because of its biological compatibility, and its oxidation potential in neutral and alkaline medium is more negative compared to the other metals, and therefore has been extensively examined. However, the limited supplies of gold in nature and exorbitant price have prevented it from being an efficient commercial catalyst. Therefore, a nanostructure Au material would be an effective way to reduce the Au loadings and to enhance the electrocatalytic performances.¹⁵,¹⁶

To date, a variety of approaches have been developed to achieve gold nanomaterials with different morphology. Most existing preparations of Au materials are focused on chemical synthesis methods, in which some capping agent is often chosen
to prevent agglomeration among particles.\textsuperscript{17} The use of a capping agent might cause the surface contamination of nanomaterials, resulting in a decreasing of the electrocatalytic activity of Au nanoparticles for the blocking of active sites. Thus the development of a simple approach to make Au nanoparticles with a clean surface on the electrode for glucose nonenzymatic sensing still has great challenges.

In this study, it is an attractive way for fabricating gold nanoclusters (GNCs) using pulse laser deposition (PLD) without any templates, surfactants, or stabilizers. It is well known that PLD is an effective approach for fabricating nanoclusters and film on a substrate surface, which has many advantages, such as a simple and direct preparation process, tight attachment of the obtained nanomaterials on the substrate, and easy cleaning treatment.\textsuperscript{18–21} In previous work, Dolbec \textit{et al.} studied the growth dynamics of pulsed laser deposited Pt nanoparticles; they also demonstrated that changing the laser kinetic energy results in Pt nanostructures of different morphology.\textsuperscript{24–26} Gonzalo \textit{et al.} reported the competing process between growth, implantation, and sputtering effects during the production of metal nanoparticles (NPs) by PLD, and concluded that the high kinetic energies involved give rise to a regulation of the NP size through self-sputtering of the deposited material.\textsuperscript{23} Some researchers have also compared nanostructure growth by PLD with other deposition methods. These works point out that the background gases and kinetic energies involved in the PLD process have an influence on the thickness and size of the obtained materials.\textsuperscript{22–26}

To evaluate the influence of the laser parameters, such as the pulse number and gas pressure, the synchronous transmission electron microscopy (TEM) images were used to observe the morphology evolution, and thus to optimize the experiment parameters. The Cu TEM grids were covered by a thin formvar/carbon copper grid film fastened to the sample stage together with the glassy carbon (GC) plates, so could obtain gold nanoclusters on the Cu grid surface simultaneously.\textsuperscript{12,27} From the TEM images, it would be found that Au nanoclusters were uniformly distributed on the surface of GC. The electrochemical tests showed that GNCs modified glassy carbon electrodes obtained in the optimized condition had high catalytic activity for glucose oxidation and strong resistance for the normal interferences, such as ascorbic acid (AA), uric acid (UA) and 4-acetaminophen (AP). The PLD method verified a relatively simple and effective nanofabrication technique to obtain GNCs with a clean surface and high catalytic properties for a glucose biosensor.

**Experimental**

**Materials**

Au target (99.99% purity, size 10 mm × 10 mm × 1.0 mm) was obtained from BAS. Co., Ltd. AA, UA, AP, ethanol, Na\textsubscript{2}HPO\textsubscript{4}·12H\textsubscript{2}O, NaH\textsubscript{2}PO\textsubscript{4}·3H\textsubscript{2}O, concentrated sulfuric acid (H\textsubscript{2}SO\textsubscript{4}) and glucose were obtained from Sinopharm Chemical Regent Co., Ltd. All of the chemicals were of analytical grade. Phosphate buffer solution (PBS, 0.1 M, pH 7.4) was made up from Na\textsubscript{2}HPO\textsubscript{4}·12H\textsubscript{2}O and NaH\textsubscript{2}PO\textsubscript{4}·3H\textsubscript{2}O. Solutions of glucose, AA, UA and AP were prepared using PBS before the electrochemical test. Glassy carbon plates (size 10 mm × 10 mm × 1.0 mm) and gold bulk electrodes (diameter, 2.0 mm) were ordered from BAS Co., Ltd. The GC plates were polished sequentially with alumina slurries of 0.3 and 0.05 μm to create a mirror finish; then, the electrodes were washed sequentially in acetone, ethanol and pure water with sonication each for 10 min and dried with nitrogen gas. In all of the procedures, we used pure water prepared with a Kertone Ultrapure Water System P60-CY (Kertone Water Treatment Co., Ltd; resistivity, > 18 MΩ·cm).

**Apparatuses**

The laser that we used was produced by Coherent Inc., Germany (Lambda Physik COMPEx PRO 205 F, λ = 248 nm, KrF). The vacuum deposition system was manufactured by the Chinese Academy of Sciences, Shenyang scientific instruments Co., Ltd. The size and morphology of the Au nanoclusters attached to the glassy carbon surface was characterized with a transmission electron microscope (TEM; FEI TECNAI20, USA). All electrochemical measurements were conducted with a 550 electrochemical workstation (Gaoss Union Instrument Co., China) at room temperature in a conventional three-electrode system, with GNCs modified GC as the working electrode, a Pt wire counter electrode and an Ag/AgCl reference electrode.

**Procedures of deposition GNCs on the surface of GC**

The depositions were performed in a high-vacuum chamber with a residual pressure lower than 4.0 × 10\textsuperscript{−4} Pa. A KrF excimer laser operating at 248 nm and a pulse length of 20 ns was used to ablate a rotating metal Au target in the vacuum chamber. The substrate was positioned with its surface parallel to the Au target at a target-to-substrate distance of 50 mm, and the temperature (295 K) of the substrates was kept fixed. Ablated material was deposited on GC plates and Cu TEM grids (Beijing Xinxing Brain Technology Co., Ltd.). The laser energy was kept fixed at 200 mJ and the repetition rate was 5 Hz. A set of samples were grown in a controlled atmosphere at Ar pressures (p\textsubscript{Ar}) of 0, 30, 70 and 100 Pa while keeping the laser number fixed at 5000. Another set was grown at fixed p\textsubscript{Ar} of 70 Pa, which changing the laser number from 5000 to 30000.

**Fabrication of GNCs/GC electrodes**

To evaluate the electrochemical properties of the GNCs directly deposited to GC (GNCs/GC), a handmade GNCs/GC working electrode was fabricated as follows. At first, a piece of GC on which GNCs were uniformly distributed on the surface of GC. The electrochemical tests showed that GNCs modified glassy carbon electrodes obtained in the optimized condition had high catalytic activity for glucose oxidation and strong resistance for the normal interferences, such as ascorbic acid (AA), uric acid (UA) and 4-acetaminophen (AP). The PLD method verified a relatively simple and effective nanofabrication technique to obtain GNCs with a clean surface and high catalytic properties for a glucose biosensor.

**Results and Discussion**

**TEM observation of the GNCs/GC surface**

First, we would like to show how the morphology of GNCs were influenced by the different inert gas pressures. Irissou \textit{et al.} has verified that the transition from structure to the other depends on the nature of the background gas and the gas pressure.\textsuperscript{24} In the case of Ar, the deposition pressure determines the velocity of the Au plasma impinging on the substrate, and hence affects the type of structure obtained. For high kinetic energies the films are highly oriented, and for low energies the morphology is nanocrystalline.\textsuperscript{25} Figure 1 shows typical TEM images of the sample surfaces deposited in an Ar gas atmosphere at different pressures with a fixed laser number of 5000. The pictures were taken from samples deposited on amorphous-C supported Cu grids. Figure 1A shows TEM images of Au films
deposited at an Ar pressure of 0 Pa; the films appear dense and compact, with no apparent pore. As the pressure is increased to 30 Pa, the TEM image reveals the appearance of an islands structure (shown in Fig. 1B). The elongated shape of most of such islands with rough edges indicates that they result from the coalescence of considerably smaller, nearly spherical particles. A similar morphology was observed for a sample grown at 70 Pa, as shown in Fig. 1C. The difference is only the size of the islands. When looking at the surface of the sample deposited at a pressure of 100 Pa (Fig. 1D), the surface morphology appears to be quite different. The surface is mostly covered by a random distribution of isolated oval-shaped particles, the sizes which are around 10 nm.

Figure 2 shows TEM images acquired from a sample deposited at 70 Pa of Ar using 5000, 10000, 20000 and 30000 laser pulses, respectively. The morphology of Au nanoclusters grown at different laser pulses is very similar. All of the sample surfaces show evidence for the presence of Au nanoclusters whose size increases as a function of the pulse number. The surface of sample obtained with 5000 and 10000 pulse number consists of a network of irregularly shaped Au island structures (Figs. 2A and 2B). With increasing the pulse number more, Au plasma was crowded onto the substrate surface, which appears darker in the picture (Fig. 2D). It is worth noticing that the Ar pressure and pulse number well differentiated the morphologies of the deposited GNCs, which would affect the properties, such as the electrical, mechanical and catalytic activity.

From the above TEM characterization, the morphology of GNCs is greatly affected by the Ar pressures and the pulse number. It is known that during plasma expansion, isolated, sphere-like nanoparticles grow and land onto the substrate. After this stage, if deposition goes on, spontaneous self-assembling of nanoparticles on the substrate leads to the formation of islands with progressively larger size and irregular shape. In the case of Ar, the gas will confine the plasma in a smaller volume by increasing the number of collisions between the plasma and the gas molecules, which in turn reduce the velocity of the neutral gold species impinging on the substrate and hence the type of structure obtained. It has been demonstrated that deposition under vacuum conditions yields dense films, whereas an inert gas environment favors the synthesis of nanoparticles with controlled size. At a fixed Ar pressure, when we increased the pulse number, the amount of Au species attached to the surface of GC has also become more.

**Electrochemical characterizations of the GNCs/GC electrodes**

For evaluating the electrochemical properties of GNCs modified GC plates, cyclic voltammograms (CV) of the GNCs/
GC electrodes \((p_{Ar} = 0, 30, 70, 100 \text{ Pa})\), the pulse number of 5000) were carried out in a 0.5 M sulfuric acid aqueous solution (Fig. 3A). The typical anodic oxidation peak starting from 1.1 V and the cathodic reduction peak appearing at 0.9 V are attributed to the formation of gold oxide, and subsequently its reductive reaction, respectively. From Fig. 3A, it was found that when the Ar pressure was at 0 Pa and 100 Pa, the value of the reduction peak was lower compared with \(p_{Ar} = 30\) and 70 Pa. An explanation for this maybe that at lower Ar pressure, that is, under the vacuum condition, the plasma from the Au target ablation would have a bigger kinetic energy, and could reach the substrate more easily. The excess gold atoms formed a smooth dense membrane, thus reducing the active surface area, and hence reducing the peak. So as to improve the Ar gas pressure to 100 Pa, a very small amount of the plasma could reach the GC surface, also could not form the island structure, and thus could not supply a larger active surface area; this dropped the cathodic peak values of the GNCs/GC electrodes.29,31,32

Figure 3B shows CV of the GNCs/GC electrodes with different pulse number; for all other experimental conditions, such as the laser energy of 200 mJ, the laser frequency of 5 Hz were kept fixed. The reduction peak currents increased with the increase of the pulse number, which reflects the increasing active sites due to the attachment of GNCs, consistent with the SEM observation stated above.

To make more evident experimental phenomena, we carried out glucose oxidation in the alkaline environment (0.1 M NaOH). Figures 3C and 3D are the CV in 0.1 M NaOH containing 10 mM glucose of GNCs/GC electrodes prepared under different experiment conditions. It is can be found that the obtained electrodes have a similar catalytic trend for glucose oxidation, so result in almost the same CV curves. From Fig. 3C, we know that increasing the Ar pressure contributes to the formation of a nanostructure, and thus increasing the catalytic activity, which results from the extended electrochemical active surface area. However, continuously increasing the Ar pressure may limit the Au plasma reaching to the GC plate, thereby reduce the activity. Therefore, GNCs deposited at the \(p_{Ar}\) of 70 Pa exhibited the highest electrocatalytic ability to glucose oxidation. Figure 3D shows the influence of the pulse number. Increasing the pulse number contributes to an enhancement of the electrocatalytic activity, while considering the dosage of gold; a pulse number of 20000 is the best experiment condition.

The TEM characterizations and electrochemical tests demonstrated that the GNCs deposited at a laser energy of 200 mJ, \(p_{Ar} = 70 \text{ Pa}\), a laser frequency of 5 Hz and a pulse number of 20000 have the best properties that we need. Therefore, we investigated the enhanced electrochemical performance of the GNCs/GC electrode, as shown in Fig. 4.

Fig. 2 TEM images showing the morphologies of GNCs on a GC surface at different pulse numbers: (A) 5000, (B) 10000, (C) 20000, and (D) 30000.
Cyclic voltammograms of the GNCs/GC electrodes were carried out in 0.5 M sulfuric acid aqueous solution compared with both the pure gold bulk electrode and a blank GC electrode (Fig. 4A). The GNCs/GC electrode shows similar electrochemical response to the bulk Au electrode, but the current value is much higher. No response was observed on the blank GC electrode. This confirms the GNCs attachment on the GC surface. For investigating the catalytical properties of GNCs, we examined the electrocatalytical oxidation of glucose on GNCs/GC electrodes. Figure 4B shows CV of a 10 mM glucose solution with different electrodes: a GC electrode, a pure gold bulk electrode and a GNCs/GC electrode, respectively. The potential sweep was performed from −0.4 to 0.7 V. However, no response appearing on the pure GC electrode, weak oxidation peaks are observed for the bulk gold electrode. Two peaks appear at −0.1 and 0.4 V, which could be ascribed to the adsorption of glucose on the electrode surface and the formation of gluconolactone and its continuous oxidation, respectively. The Au substrate had a similar peak position and shape for glucose oxidation, but exhibited a different peak current (Fig. 4C). The peak current of a GNCs modified GC electrode was about 32 μA, which is 8 times that of the bulk gold electrode (4 μA). This indicates that the dense attachment of GNCs on the GC electrode enhances the catalytic activity for glucose oxidation compared to the bulk Au. The gold oxide was formed on the surface after 0.4 V, which passivated the active surface of Au, and causing a decrease of the oxidation peak. In cathodic scanning, a fresh Au surface is released by the reduction of Au oxide. Thus, another oxidation peak appears at a potential of about 0.35 V.33,34 Then, the CV performance of the GNCs modified GCE was investigated with and without the presence of 10 mM glucose. When glucose was added, the anodic current increased significantly compared with without adding glucose. This directly illustrates that the oxidation peak is formed by the electrocatalytic oxidation of glucose. We also studied the multi-times CV test in a 0.1 M PBS solution containing 10 mM glucose and found that the oxidation peak current for the 50 cycles was just reduced by less than 5% compared with the first time. There was a little change of the peak shape and the peak position. This indicates that the material we obtained was very stable for the oxidation of glucose, reflecting good stability and repeatability of the GNCs modified GCE.

The detection potential that affected the amperometric detection of glucose needed to be investigated. Figure 5 shows a comparison of the amperometric responses of the GNCs modified GCE at different potentials, ranging from 0.20 to 0.30 V with successive addition of 1.0 mM glucose to the stirred PBS solution. When the glucose was successively added into the solution, the current increased steeply to reach a stable value; the response time was within 2 s. We could see that, at a potential of 0.30 V, the amperometric response was stable, but the sensitivity was not so good because of the formation of Au.

Fig. 3 Cyclic voltammograms (CV) of GNCs/GC electrodes prepared under (A) different Ar gas pressures and (B) different pulse numbers in a solution of 0.5 M H2SO4; (C), (D) is the CV in 0.1 M NaOH containing 10 mM glucose of GNCs/GC electrodes prepared under different Ar gas pressures and different pulse numbers, respectively. Scan rate, 100 mV s⁻¹.
oxide. At a potential of 0.20 V, the sensitivity was higher than 0.30 V, but not the best due to the insufficient potential. While at potentials of 0.25 V, the signals were sensitive and stable. The amperometric response was also tested at low potentials, such as 0.1 and 0.15 V. Steady signals could be found, but the linear range was narrow, which might be due to the incompletely oxidation of glucose at a low potential and the gathering of intermediates on the surface of electrode, which blocked some of the active sites. To evaluate the influence of electro-active species toward the detection of glucose at the selected detection potential of 0.25 V, amperometric detection was performed, as shown in the Fig. 6A. The results show that the response to the addition of glucose is remarkable, while the effect derived from AA, AP, and UA is not obvious. This indicated nonenzymatic glucose detection at the proposed electrode can be performed at 0.25 V without any significant interference from AA, AP, and UA.

In the evaluation of an amperometric sensor, current responses were measured under different concentrations of the objective analyte at a fixed potential and at fixed times. Figure 6B shows the typical steady state amperometric response of the GNCs/GC electrode to the successive addition of glucose in solution of PBS (pH 7.4) at an applied potential of 0.25 V. In the electrochemical test, the successive addition of 10.0 μL, 1.5 M glucose to the solution (final volume 15.0 mL) was performed at an applied potential of 0.25 V. The corresponding electrochemical response was recorded while the solution was stirred constantly. The response displays a linear range from 1.0 to 20 mM (every addition of glucose concentration was 1.0 mM) with a correlation coefficient of 0.991. The sensitivity was evaluated to be 31.18 μA mM⁻¹ cm⁻² (current response of
1 mM glucose divided by the electrode geometric area, 0.0314 cm²), as shown in Fig. 6B. The upper-limit of the linear range is far beyond the physiological level (3 – 8 mM), suggesting that the electrode developed here would be useful in the detection of the glucose concentration at physiological levels. The detection limit is estimated to be ca. 0.05 mM at a signal/noise (S/N) of 3, and the response time is about 2 s. Indeed, the advantages of the proposed glucose sensor were obvious compared to the traditional sensors based on GOx, such as the higher sensitivity and stability.35,36 Moreover, compared to other similar nonenzymatic glucose sensors fabricated by noble metal nano materials, the developed GNCs also demonstrated good sensing performance in terms of sensitivity, detection potential and linear range, which meant that the GNCs are promising for nonenzymatic glucose sensing.37,38

Effect of the interferences and stability of the electrode on the detection of glucose

As mentioned previously, one of the main challenges in the nonenzymatic glucose detection of glucose is interfering electrochemical signals caused by some coexisting organic substances, such as AA, UA, and AP. Therefore, the effect of the interferences was investigated for the GNCs/GC electrode. As can be seen in Fig. 7A, adding typical interfering molecules in blood, such as AA, AP, UA, etc. could not bring any apparent change comparing the oxidation of glucose on the GNCs/GC electrode, which is consistent with the results of Fig. 6A. This is another advantage of the GNCs as a new catalyst with good selectivity in the fabrication of nonenzymatic glucose biosensors.

For real applications of the Au electrocatalyst, the long-term electrocatalytic activity and stability are of great importance. The CV of GNCs modified GC will become similar and stable after multiple cycles. There were 2.14 and 3.32% declines in the electro-oxidation current after 30 and 50 cycles, respectively, which illustrated that the adsorption of GNCs on the GC surface is very tight (Fig. 7B). The long-term stability of the nonenzymatic sensor was evaluated through the current response of 10 mM glucose recorded at intervals over 20 days, and the GNCs/GC electrode was stored in air when not in use. The results indicate that the sensor used retains more than 90% of the initial current in continuous tests (Fig. 7C), suggesting that the non-enzymatic glucose sensor has favorable long-term stability.

Conclusions

Au nanoclusters were successfully fabricated on the surface of
the GC plates by PLD. The morphology and size of the GNCs could be easily tuned through the Ar gas pressures and the pulse numbers in the deposition process. The GNCs attached on the electrodes are very clean without any other contaminations, which could significantly promote the electrocatalytic ability toward the oxidation of glucose. The GNCs/GC electrodes show a wide linear range from 0.1 to 20 mM with a sensitivity of 31.18 μA cm⁻² mM⁻¹ and a detection limit down to 0.05 mM for the detection of glucose. Common interfering species naturally present in the physiological environment have no obvious effects for the oxidation of glucose on GNCs/GC electrodes. The good electrocatalytic ability, high selectivity and excellent stability all together imply that GNCs could work as excellent catalyst materials in the fabrication of nonenzymatic glucose biosensors. Furthermore, the present approach might be extendable to the immobilization of other NPs on certain substrates with different sensing and catalysis applications.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (Grant Nos. 51102085, 61274010, and 51202062), Program for New Century Excellent Talents in University, Ministry of Education of China (NCET-09-0135), Natural Science Foundation of Hubei Province (Nos. 2011CDB057 and 2011CDA81), Science foundation from Hubei Provincial Department of Education (No. Q20111002), Wuhan Municipal academic leaders program (200951830550) and the Plan of Youth Science and technology, Wuhan City (2014072704011250).

References