Preparation of Pd-Pt alloy nanoparticles/nanoporous gold/graphene paper electrode and its use for real-time detection for glucose and \( \text{H}_2\text{O}_2 \) from living cells

Abstract: Graphene (GR) and Carbon nanotubes (CNTs) are high-performance carbon non-materials. Their excellent electron transfer and electrocatalytic properties have attracted much interest in the field of electrochemistry. In this work graphene as a nanohybrid composites based on noble metal/metal alloy were applied to study the electrochemical sensing of hydrogen peroxide and glucose. The main contents were described as follows. We have developed the design and synthesis of a new type of high-performance nanocatalysts, i.e. deposit tiny palladium and platinum alloy nanoparticles on the surface of free-standing ultrathin nanoporous gold film (Pd&Pt@NPG), and transfer it to the surface of graphene paper for the fabrication free-standing and flexible Pd&Pt@NPG/graphene paper, which enable to be used for real-time tracking the secretion of \( \text{H}_2\text{O}_2 \) in different types of living human cells.

Keywords: Graphene, metal alloys nanoparticles (Au, Pt, Pd), hydrogen peroxide, Glucose, Electrochemical sensors.

INTRODUCTION

Due to its strong oxidizing property, hydrogen peroxide (H\( \text{O}_2 \)) is widely used in the pharmaceutical, chemical and biochemical industries (Guo, S. et al., 2009; Choudhury, N. A. et al., 2005; & Miley, G. H. et al., 2007). The quantitative determination of H\( \text{O}_2 \) has become extremely important in recent years. Many methods such as titrimetry (Klassen, N. V. et al., 1994), spectrometry (Tanner, P. A., & Wong, A. Y. S. 1998), chemiluminescence (Diaz, A. N. et al., 1998) and electrochemistry (Cui, K. et al., 2008) have been developed to detect H\( \text{O}_2 \). Electrochemical methods have attracted much attention recently due to their low cost and high sensitivity (Lian, W. et al., 2009; & Chun Song, X. et al., 2012).

Different nanoparticles have been widely and successfully used to construct electrochemical sensors. For example, Graphene-CdS nanocomposites have been successfully prepared by in situ growth of CdS nanocrystals on the surface of graphene sheets, which show strong and stable electrochemiluminescent emission when they react with H\( \text{O}_2 \) (Wang, K. et al., 2010). The Ag nanoparticles electrode was directly fabricated with a simple single step electrochemical deposition method, and the developed Ag nanoparticles sensor had excellent electrocatalytic activity for H\( \text{O}_2 \) detection (Song, X. C. et al., 2011). Single crystal and vertically aligned cobalt oxide nanowalls were synthesized by directly heating Co foil on a hot plate under ambient conditions which showed a fast response and high sensitivity in the amperometric detection of H\( \text{O}_2 \) (Jia, W. et al., 2009). CuO nanoflowers electrode, with excellent electrocatalytic activity, large surface-to-volume ratio and efficient electron transport property, has enabled stable and highly sensitive performance for the nonenzymatic H\( \text{O}_2 \) sensor (Song, M. J. et al., 2010). Diabetes mellitus is a worldwide public health problem. The metabolic disorder, resulting from insulin deficiency and hyperglycemia, is reflected by glucose concentration higher or lower than the normal range (4.4–6.6 \( \mu \)M) (Wang, J. 2008). Hence development of glucose sensor with high performance is very important for the diagnosis of some diabetes mellitus. Glucose electrochemical sensor has been widely used because of its rapid, accurate, selective and low cost or other potential applications. The electrochemical glucose enzymatic sensor can perform high selectivity and sensitivity due to specificity of enzyme. But the most common and serious problems with enzymatic glucose sensors are insufficient long-term stability and unsatisfactory reproducibility originating from the nature of the enzymes as well as the activity of the immobilized enzymes (Park, S. et al., 2006).
In addition to temperature, pH, and toxic chemicals for sterilization, the responses of glucose sensors are a function of humidity. Both high and low humidity may be significantly harmful to the sensors in use as well as storage. Therefore, the poor stability of enzymatic sensors and the interference of some electro-oxidizable species remain as problems for application (Feng, D. et al., 2009). So preference is given to non-enzymatic sensor which means the direct electrochemical oxidation of glucose without enzyme. Nonenzymatic electrode would exhibit conveniences and advantages to avoid the drawbacks of enzymatic sensors. Dealloyed Au/Ag alloy membranes have a three-dimension nanoporous structure, consisting of gold ligaments and nanopore channels (Fujita, T. et al., 2008). Hence, the dealloyed nanoporous gold (NPG) possesses high electric conductivity and a large surface area, which offer a large number of adsorption sites for proteins and enzymes and could increase the signal-to-noise ratio in the miniaturization systems (Yu, C. et al., 2007). Furthermore, NPG has excellent stability and biocompatibility for use in biosensors and reactors (Sotiropoulou, S. et al., 2005). The striking properties of the nanoporous gold have motivated intensive interest in their utilization in biosensor designs. In this work, we reported for the first time the design and synthesis of noble type of NPG-based nanocatalyst, i.e. deposit tiny palladium and platinum alloy nanoparticles on the surface of free-standing ultrathin nanoporous gold film (Pd&Pt@NPG/GP) on graphene paper by a simple one-step method, and explored its practical application as robust electrode materials in electrochemical sensor for the nonenzymatic detection of glucose and hydrogen peroxide.

RESULTS AND DISCUSSION

Physical Characterization of NPG and Pd&Pt@NPG

NPG is formed from Au50Ag50 alloy membrane (weight ratio) by 30min dealloying process on concentrated nitric acid and subsequently maintained on Ultra-pure water. Chloroplatinic acid hydrate and palladium chloride acid and formic acid are mixed and dispersed under 25 °C water bath. Then, NPG is carefully transferred to the mixed solution. After this process, the reaction container is kept out of light and under 25 °C water bath for 3 h to obtain Pd&Pt@NPG (Figure 1).

Figure 1. Illustrative fabrication process of the Pd&Pt@NPG/graphene paper electrode for the biosensor application.

The scanning electron microscopy (SEM) images in (Figure 2.A) shows that ligaments and nanoporous channels are formed uniformly across the entire NPG with ligament size vary from 10 to 50 nm, and have the highest possibility to be about 30 nm, highly consistent with the previous reports (Yan, M. et al., 2012). Surface morphology of free-standing NPG film is also investigated by the transmission electron microscopy (TEM) image, showing that the NPG film has a typical three-dimensional porous structure (Figure 3.A). Pd&Pt-NPs are decorated on NPG via reducing PdCl42- and PtCl62- by HCOOH at mild temperature. After reaction of 3h, the surface of Pd&Pt@NPG film has been randomly covered by a layer of tiny Pd&Pt-NPs (Figure 3.B), and the surface of NPG film becomes very rough (Figure 3.B). The HRTEM image of Pd&Pt@NP (Figure 3.C), besides, 3.3D is the HRTEM image which illustrates the lattice spacing’s of each element.
Figure 2. SEM images of (A) NPG obtained by 30 min dealloying treatment; (B) Pd&Pt@NPG, which are delivered as a reference to the TEM images in large scale.

Figure 3. TEM images of (A) NPG and (B) Pd&Pt@NPG, (C) is the HRTEM image of Pd&Pt@NPG. Besides, (D) is the HRTEM image which illustrates the lattice spacings of each element.

XPS analysis of Pd&Pt@NPG samples was performed to identify chemical species present on the surface of the composites. High resolution XPS spectra of the Pt4f, Pd 3d and Au 4f regions are shown in Figure 4. The spectrum is composed of the Pt 4f7/2 and Pt 4f5/2 peaks having a binding energy difference of 3.33 eV. The Pt 4f7/2 and Pt 4f5/2 peak was deconvoluted into two and three component peaks, respectively, i.e. component peaks A and B for the Pt 4f7/2 peak, and component respond to the Pd 3d5/2 and Pd 3d3/2 in...
palladium nanoparticles (Jaramillo, T. F. et al., 2003). The Au 4f7/2 peak appeared at a binding energy of 84.1 eV and the Au 4f3/2 peak appeared at 87.7 eV, confirming the formation of metallic gold (Oesch, U., & Janata, J. 1983). Besides the composition, we obtained the elements content of the catalyst, as show in Table 3.1. It is clear that the atomic ration of Pd&Pt-NPs (ac. 27%) is much smaller than that of Au-NPs (ac. 73%).

Figure 4. (A, B, C) XPS spectra of the Pt 4f, Pd 3d and Au 4f of Pt&Pd@NPG.

| Table 1: Elements content of Pt&Pt@NPG catalyst from XPS analysis. |
|-----------------|-----|-----|-----|
| Element         | Pd  | Pt  | Au  |
| Atomic ratio (%)| 6.4 ±0.5 | 20.3±0.9 | 73.3 ±1.4 |

Electrochemical characteristics of modified electrodes

In order to electrochemically characterize the real surface of the fabricated electrodes, a cyclic voltammogram was obtained in 0.5M H2SO4 solution using a scan rate of 50 mV/s. The applied potential ranged from -0.2 to 1.4 V vs. SCE. The RF was calculated as laid out in (McNaught, A.D., & Wilkinson, A. 1986):

Roughness factor (RF): \( f_r = \frac{A_r}{A_g} \)

Where \( A_r \) is the real (true, actual) surface area and \( A_g \) is the geometric surface area. The real surface area (\( A_r \)) of Pd&Pt and Au can be theoretically calculated from the reduction peaks of their oxide species, which is more practical than using the hydrogen adsorption/desorption peaks because of the relatively higher charges involved (Park, I. S. et al., 2007; &...
Friedrich, K. A. et al., 1998). The calculation equation is:

$$A_r = \frac{\int u}{a \cdot v} \quad (2)$$

$I$ and $U$ are obtained from data of CVs. $v$ is the scan rate of the test. $a$ is 435 $\mu$Q cm$^{-2}$ and 400 $\mu$Q cm$^{-2}$ for Pd&Pt and Au, respectively as shown in figure 5 A, the reduction peak for Pd&Pt and Au is 0.36 V and 0.88 V respectively, which are greatly in accordance with previous reports (Park, I. S. et al., 2007; & Friedrich, K. A. et al., 1998). The real surface areas and roughness factors are shown in table 2. The roughness factor of Pd&Pt@NPG (59.98) is much higher than that of NPG (5.25), which indicate its excellent catalytic activity of the Pd&Pt@NPG/GP catalyst in selectively enhancing the faradic current of kinetically controlled sluggish reactions. To further understand the electrochemical performance characteristics, we resorted to electrochemical impedance spectroscopy (EIS) carried out at open circuit potential with an a perturbation of 5 mg in the frequency range of 1000 kHz to 0.01 Hz. Figure 5. B shows the Nyquist plots, thus obtained. The EIS data were fitted based on an equivalent circuit model consisting of bulk solution resistance $R_s$, charge-transfer resistance $R_{ct}$, double-layer capacitance $C_{dl}$, and Warburg resistance ($W$), and the result is shown in Figure 5.B, the conductivity of pt&Pd@NPG@GP is very close to NPG/GP and much higher than the bar of GP.

![Figure 5.](image)

**Figure 5.** (A) CVs of the fabricated Pt&Pd@NPG (dark line) and NPG (red line) electrodes in 0.5M H$_2$SO$_4$ solution in order to obtain the surface roughness factor. Scan rate: 50 mV/s. (B) EIS Nyquist plots of GP, NPG@GP and Pd&Pt@NPG@GP electrodes (inset: equivalent circuit diagram proposed for analysis of the EIS data)

| Table 2: The real surface areas and roughness factors of NPG and Pd&Pt@NPG samples. |
|---------------------------------|-----------------|-----------------|----------------|
| **Sample** | **Pd&Pt $A_r$ (cm$^2$)** | **Au $A_r$ (cm$^2$)** | **RF** |
| NPG | --- | 0.37 | 5.25 |
| Pd&Pt@NPG | 4.42 | --- | 59.98 |

Ps: The geometric surface area is 0.07069 cm$^2$.

| Table 3: Rs and Rct of NPG/GP and Pd&Pt@NPG/GP electrodes, bare GP electrode is also included for comparison. |
|---------------------------------|---------------|---------------|
| **Sample** | **Rs/ohm** | **Rct/ohm** |
| GP | 19.5 | 109 |
| NPG@GP | 18.7 | 89 |
| Pd&Pt@NPG@GP | 18.6 | 93 |

**Electrocatalytic activity of modified electrode toward glucose detection**

Figure 6.A depicts the CVs of Pd&Pt@NPG/graphene paper electrode in the absence and presence of 5, 10, 20 and 50 mM glucose in 0.1M PBS (pH=7.4). We can clearly see that the redox current is increased with the glucose concentration increase. For comparison, the NPG electrode is also investigated in the 0.1M PBS + 10mm glucose solution, and due to the absence of Pd&Pt-NPs/graphene paper in the surface of NPG, there is nearly no redox peaks in the CV curve, which indicates the highly enhanced catalytic property.
of Pd&Pt@NPG/graphene paper. Multiple anodic peaks attributed to the oxidation of glucose and resulting intermediates are observed for the positive scan. During the cathodic potential scan, the oxidation of glucose is suppressed in the high potential range because of the presence of surface oxide. With the reduction of Pt oxide, more surface-active sites are available for the oxidation of glucose, resulting in large and broad oxidation peak. These results can be explained by a well-accepted mechanism of glucose oxidation on a Pt electrode in neutral media (Habrioux, A. et al., 2007). These results explain and indicate that Pd&Pt@NPG/graphene paper can effectively catalyze glucose oxidation on the modified electrode surface. Figure 6.B shows the typical amperometric responses of the Pd&Pt@NPG/graphene paper upon the successive injection of glucose into the PBS solution (pH=7.4). Upon each addition of glucose, the response of the Pd&Pt@NPG/GP electrode based-sensor rapidly reaches 95% of the steady-state value within 3s. A good linear dependence on the glucose concentration in the range of 0.1-20mM was achieved, with a sensitivity of 39.05 µA mM⁻¹ cm⁻² and a detection limit down to 50 µM at a signal-to-noise (S/N) ratio of 3 (Figure 6.C).

Figure 6. (A) CV curves of Pt&Pd@NPG/graphene paper electrode in 0.1 M PBS (pH 7.4) in the absence (blank line) and in the presence of 5, 10, 20 and 50 mM glucose. Inset is the CV curves comparison of NPG/graphene paper (dark line) and Pt&Pd@NPG/graphene paper (Green line) electrodes in 0.1M PBS + 10mM glucose solution. Scan rate: 50mVol. (B) Amperometric response of Pt&Pd@NPG/graphene paper electrode to the successive addition of 0.1, 0.2, 0.4, 0.6, 0.8, 1.0, 2.0, 4.0, 6.0, 8.0 and 10.0 mM glucose in PBS buffer (pH 7.4) under magnetic stirring; inset shows the amperometric response of Pt&Pd@NPG/graphene paper electrodes at a lower concentration detected. (C) Corresponding calibration curve. Applied potential: 0.30V.

**Amperometric response for H₂O₂ detection**

Electrochemical reduction of H₂O₂ by the Pd&Pt@NPG/graphene paper was studied. Figure 7.A shows the typical cyclic voltammograms (CVs) of H₂O₂ reduction by Pd&Pt@NPG/GP in 0.1 M PBS (pH=7.4) containing 5 mM H₂O₂, as a comparison. We can clearly see that, with the present of H₂O₂ concentration, the Pd&Pt@NPG/GP generates reduction current peak, and without of H₂O₂ concentration nearly no obvious current peak was detected. This infers that Pd&Pt@NPG/GP catalyst has a robust electrochemical reduction of H₂O₂.

The H₂O₂ detection sensitivity of the Pd&Pt@NPG/GP was examined by a current-time (i-t) technique at a constant potential. Figure 7.B and the inset show the typical amperometric responses of the Pd&Pt@NPG/GP electrode upon the addition of H₂O₂ into the stirring 0.1 M PBS (pH=7.4) at 0.25 V. As H₂O₂ was added, the modified electrode responded rapidly to the substrate, achieving the maximum steady-state current within 2s. This fast electrode reduction response can be attributed to the fact that H₂O₂ is rapidly absorbed and activated on the surface of the Pd&Pt@NPG. The current vs. H₂O₂ concentration is drawn in Figure 7.C. The amperometric response of the Pd&Pt@NPG/GP electrode to the successive additions to H₂O₂ shows that the rapidly equilibrated current is proportional to the concentration of H₂O₂ in the range of 0.1-26370 µM, with a sensitivity of 2.37mA mM⁻¹ cm⁻². And the detection limit is down to 0.1µM based on S/N=3. This novel type of biosensor has outstanding analytical performance in sensing H₂O₂, which make it useful in different detection systems for in vitro and in vivo H₂O₂ detection. Anti-interference property is also an important parameter for H₂O₂ sensors.
Due to the outstanding performance of high sensitivity, wide linear range and anti-interference property, the proposed Pd&Pt@NPG/graphene paper based biosensor was used for in vitro tracking H$_2$O$_2$ secretion by live cells. In this work, three types of living human cells, i.e., Human cervical carcinoma HeLa cell, RSC96 Schwann cells and Human Umbilical Vein Endothelial cells (HUVEC) were chosen as the probes for real-time electrochemical determination of H$_2$O$_2$ released upon being stimulated. Our results demonstrate that after being incubated with the Pd&Pt@NPG/graphene paper for over 2h, the HeLa cells, RSC96 cells and HUVEC cells still maintained their viability and activity, and did not show any changes in cell morphology and viability, as shown in the bright-field and dark-field microscope image (Figure 8. A-F), indicating the good biocompatibility of Pd&Pt@NPG/graphene paper electrode. The cells of 80% confluency are induced to release H$_2$O$_2$ by injecting N-formylmethionyl-leucyl-phenylalanine (fMLP), which is one of the synthetic N-formyl methionyl peptides that can stimulate the secretion of several effectors molecules including interleukin 1, lysosomal enzymes, tumor necrosis factor, nitric oxide and reactive oxygen intermediates (ROI) by living cells. Figure 8. G-I depicts the amperometric response changes of Pd&Pt@NPG/graphene paper electrode located near the living cells lines in a culture medium consisting of Dulbecco’s modified Eagle medium (DMEM) at 37°C. After the injection of 10 ul fMLP (0.1 mm), a significant decreased current is observed for all the living cells at the applied potential of 0.25 V, followed by a gradual increase of current, and finally reaches a plateau (Figure 8. J). However, the control wells containing no living cells do not generate any signal response to the addition of fMLP (Figure 8. I). These results together suggest that the generated increase of amperometric responses are ascribed to the electrochemical reduction H$_2$O$_2$ released by living cells upon being simulated by fMLP. Moreover, the increased current densities and the times to reach the plateau for these three types of living cells are investigated in details. As shown in Figure 8. K and 8. F, upon the addition of equal amount of fMLP, the increase...
amperometric current densities for Hela cells, HUVEC cells and RSC96 cells are 29, 11 and 51 µA cm$^2$. And the times to reach the plateau for these three types of living cells also increase in the order of Hela cells > RSC96 cells > HUVEC cells. Although the clear reason is still unknown at the present stage, these observations substantially demonstrate that the proposed biosensor based on Pd&Pt@NPG/GP establishes a sensitive, reliable and robust method for the qualitative determination of H$_2$O$_2$ secreted by different living cells and could potentially be useful for further physiological and pathological investigations.

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Figure 8. The bright-field images of (A): Hela cells, (B): HUVEC cells and (C): RSC96 cells. The dark-field images of (D): Hela cells, (E): HUVEC cells and (F): RSC96 cells. Amperometric responses of the Pd&Pt@NPG/GP electrode in 0.1 M PBS (pH=7.4) with the addition of 10 µM fMLP in the absent of cell, as (J) shows, and in the present of (G): Hela cells, (H): HUVEC cells and (I): RSC96 cells. Applied potential: 0.25V. (K): Increase amperometric current densities of these cells. (L): Times needed to reach the plateau of these cells.

Table 4: Comparison of the nonenzymatic glucose and H$_2$O$_2$ detection Performance

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<tr>
<th>Electrode</th>
<th>Glucose</th>
<th>H$_2$O$_2$</th>
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<tr>
<td></td>
<td>sensitivity µA/mM/cm$^2$</td>
<td>linear range mM</td>
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<tr>
<td>PtNi-NPs/graphene</td>
<td>20.42</td>
<td>Up to 35</td>
</tr>
<tr>
<td>Ni(OH)$_2$/nanoplates/RGO</td>
<td>11.43</td>
<td>0.002–3.1</td>
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<tr>
<td>Pd&amp;Pt@NPG/GP</td>
<td>39.05</td>
<td>0.1–20</td>
</tr>
<tr>
<td>Ni CFP electrode</td>
<td>3.3</td>
<td>0.02–2.5</td>
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<tr>
<td>Cu$_2$O-rGO</td>
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<td>Pd-CNFs</td>
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<td>PtPdNDs/GNs</td>
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CONCLUSIONS
In summary, we have demonstrated the design and synthesis of a new type of high-performance nanocatalysts, i.e. deposit tiny palladium and platinum alloy nanoparticles on the surface of free-standing ultrathin nanoporous gold (Pd&Pt@NPG/GP), and then transfer it to the surface of graphene paper to fabricate freestanding and flexible nanohybrid electrode. Its practical application has further been investigated by using it as robust electrode materials in electrochemical sensor for in situ molecular detection of both glucose and H2O2 secretion by living cells.

REFERENCES


