

Construction of Sandwich-like Structured GO/Cu₂O/GO Electrochemical Biosensor for Sensitive Detection of H₂O₂ Releasing from Living Cells

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Abstract

A well-designed sandwich-like structure based on Cu_2O nanocubes sandwiched between graphene oxide (GO) buffer layer was fabricated on indium tin oxide (ITO)/glass substrate via drop-casting method. The sandwich-like morphologies, GO/ Cu_2O /GO/ITO structures, were characterized by scanning electron microscopy (SEM), transmission electron microscopy (TEM) and X-ray diffraction (XRD) measurements. Electrochemical studies demonstrated the fact that GO/ Cu_2O /GO/ITO electrode revealed the superior electro-catalytic activity and electrical conductivity as a result of the introduction of graphene oxide and formation of sandwich-like structures, which effectively improved the electrical performances of Cu_2O -based sandwich structure in H_2O_2 detection. The proposed sandwich-like GO/ Cu_2O /GO/ITO sensor was experimentally optimized and displayed a widespread linear response in the range from 0.25 μM to 20 mM with a low limit of detection (LOD) of 0.2 μM (S/N=3) in H_2O_2 sensing. Besides achieving acceptable selectivity, excellent reproducibility, and long-term stability of sandwich-like lamellar structures, GO/ Cu_2O /GO/ITO sensor exhibited high performance in detecting H_2O_2 released from A549 cells. Therefore, the novel sandwich-like GO/ Cu_2O /GO/ITO sensor provides an economical, potent and high-throughput platform for detecting various species involving H_2O_2 -generation reactions for biomedical applications.

1. Introduction

Hydrogen peroxide (H_2O_2) is an important reactive oxygen species, which plays an important role in aging, carcinogenesis and other biological processes [1, 2]. Appropriate levels of hydrogen peroxide play an important role in ensuring intracellular signal transduction and maintaining normal cell functions. However, abnormal levels of H_2O_2 can induce numerous kinds of biological damages, leading to neurodegeneration, myocardial infarct, Alzheimer's disease, Parkinson's disease, and even cancers [3, 4]. Selective and quantitative detection of H_2O_2 in cells are essential for clinical diagnostics and patient monitoring. Contemporarily, numerous analytical methods including spectrometry [5], chromatography [6], fluorescence [7, 8] and electrochemistry [9] have been applied for H_2O_2 monitoring. Among these methods, the electrochemical technique is getting enormous interest owing to its simplicity, fast response and straightforward manipulation in analysis [10–12]. High detection performance and specificity are the main advantages of enzyme-based electrochemical sensors. However, how to solve the instability of enzyme is the main problem in practical application [13]. Therefore, that functional materials are explored for the development of enzyme-free H_2O_2 sensors has received significantly attention.

Because of their high catalytic activity and good stability, Nanoparticles (NPs) of metal oxide have been used as electrochemical catalysts for enzyme-free H_2O_2 sensors [14]. Metal oxides have some special physicochemical properties, such as high alkaline corrosion resistance in electrochemical environments, multiple oxidation states, and distinct crystalline structures which are especially obvious in transition-metal oxides. At present, abundant NPs have already been successfully applied to the electrochemical

detection of H_2O_2 molecules, such as Co_3O_4 [15, 16], Fe_3O_4 [17], Cu_2O [18, 19], MnO_2 [20]. It is not uncommon for Cu_2O application in photosplitting of water, solar energy conversion, photocatalysis, lithium-ion batteries, gas sensing and so on [21, 22]. Moreover, Cu_2O has been considered as a encouraging candidate for electrochemical sensor on account of its p-type semiconductor character and active electron-cavity pair system [23].

However, the instability of Cu_2O hindered their practical applications as sensing material. Therefore, rationally protective strategies are under consideration to enhance their stability. Two-dimensional (2D) graphene has the sp^2 -bonded carbon atoms arranged in a honeycomb crystal lattice. This carbon material has attracted tremendous attention due to its good biocompatibility, high electron conductivity, large specific surface and fast heterogeneous electron-transfer rate on its sheet edges [24, 25]. Thus, graphene was utilized as a robust scaffold for nanoparticles to form hybrid materials with improved properties. At present, various graphene-supported metal oxides have been used widely in electrochemical sensing and electrocatalysis. For example, Zhao et al. successfully prepared nano- Fe_3O_4 /graphene composites with enhanced lithium storage and electrocatalysis properties [26]. Abdel Hameed et al. reported the synthesis of NiO/Gr and the composite exhibited effective electrocatalysts for urea electro-oxidation in alkaline medium [27]. Deng et al. prepared reduced graphene oxide (rGO)-conjugated Cu_2O nanowires to exhibit high sensing sensitivity toward NO_2 detection at room temperature [28]. The decoration of graphene with metal oxide nanoparticles can lead to more rapid and sensitive current responses because it can provide larger electrochemical active surface areas and effectively accelerate the electron transfer between electrode and detection molecules. Apparently, the $\text{Cu}_2\text{O}/\text{graphene}$ composites have been constructed for enhancing the detection limit, sensitivity and stability in amperometric response of bioactive substances [29, 30]. The structure-induced performance therefore enlightens us on further morphological and structural design. In previous report, sandwich-like structure was conducive to component-controlled design and thus reveals the related improved and superior performance in supercapacitors [31], immunosensing [32] and Li-ion battery [33].

Herein, a novel $\text{GO}/\text{Cu}_2\text{O}/\text{GO}$ sandwich-like structure, inspired by the construction of functional lamellar structures, was therefore constructed on ITO substrate for H_2O_2 detection, which was rarely reported before. The sandwich-like lamellar structures, with ~ 20 nm Cu_2O nanocubes sandwiched between GO buffer layers, greatly enhanced the LOD and the stability in H_2O_2 detection in living cell. Therefore, a sensitive probe for extracellular H_2O_2 detection was presented, which has promising potential in physiology and pathology.

2. Experimental Section

2.1. Chemicals

All the reagents used in the experiment are of analysis level and can be used directly without purification. Copper sulfate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$), sodium hydroxide (NaOH), sodium hydrogen phosphate (Na_2HPO_4) and

sodium dihydrogen phosphate (NaH_2PO_4) were purchased from Sinopharm Chemical Reagent Co., Ltd. China. Ascorbic acid, sodium ascorbate and sodium dodecyl sulfate (SDS) was purchased from Aladdin Ltd. Graphene oxide (GO) dispersion with concentration of 2 mg/mL was obtained from XF-NANO Inc. Nafion PFSA polymer dispersion (5%) were purchased from Beijing Honghaitian technology Co., Ltd. Deionized water was used in all the experiments.

2.2. Synthesis of Cu_2O nanocubes

Cu_2O nanocubes were synthesized by reported seed-mediated pathway with minor modifications [34]. A volume of 100 mL aqueous solution containing $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (1 mM) and SDS (33 mM) was prepared with vigorous stirring at room temperature, and then 2.50 mL of sodium ascorbate (0.2 M) was introduced and stirring for 5 s. The color of precipitate quickly became yellow. Then 5 mL of 1 M NaOH was added into the mixture. The solution was stirred for another 10 s and aging for 2 h to promote growth of Cu_2O nanocrystal. The cubic size can be easily controlled by the seed-growth steps in our previous work [35]. Afterwards, the precipitates were collected by rinse-centrifugation cycles. The obtained Cu_2O nanocubes were washed with water-ethanol solution (1:1 v/v) several times and finally dried in vacuum at room temperature for 12 h.

2.3. Fabrication of GO/ Cu_2O /GO sandwich-like electrode

The indium tin oxide (ITO) glass (2 cm × 2 cm) was cleaned by sonication in acetone, ethanol and deionized water for 10 min, respectively, and then dried under nitrogen sweeping. Firstly, 5 mg of the synthesized Cu_2O nanocubes were dispersed in 5 mL 0.1% Nafion solution and the mixture was ultrasonicated for 1 h to obtain uniform solution (1 mg/mL). Secondly, 200 μL GO dispersion (0.5 mg/mL) was cautiously dripped on the surface of ITO glass and dried in air about 3 h at room temperature to form GO/ITO electrode. Thirdly, 200 μL of Cu_2O /Nafion (1 mg/mL) suspensions and GO layer were sequentially introduced in the similar way to form GO/ Cu_2O /GO/ITO sandwich-like electrode. The layer thickness varied with the amount of GO and Cu_2O , which could be controlled by the repeated times of drop-casting procedures. In controlled experiments, GO, Cu_2O and Cu_2O /GO lamellar structures were also constructed on ITO substrate via the similar process, respectively.

2.4 Instrumentation and characterization

The X-ray diffraction (XRD) patterns were obtained with Empyrean diffractometer using Cu K α radiation ($\lambda = 0.15405$ nm, 40 kV, 100 mA) from 20 to 90 degrees with continuous scanning type. The scanning electron microscopy (SEM) characterization was carried on JSM-6360LV (JEOL). The transmission electron microscopy (TEM) measurements were conducted using a JEM-1011 (JEOL). High-resolution transmission electron microscopy (HRTEM) images were measured by Tecnai G2 F30 S-Twin TEM (Fei) with energy dispersed spectrum detector. The product powders were dispersed in ethanol, and the suspensions were dropped on the copper grid for the TEM and HRTEM characterizations.

2.5. Electrochemical measurements

Electrochemical measurements were carried out with CHI 660E electrochemical workstation to study the electrochemical activities of the synthesized samples for H_2O_2 detection. All the electrochemical measurements were carried out in a conventional three-electrode system with a magnetic stirrer, containing a platinum foil (1 cm × 1 cm) as the auxiliary electrode, the modified lamellar structures on ITO substrate as the working electrode, and a Ag/AgCl (3 M KCl) electrode as the reference electrode. Human lung adenocarcinoma (A549) cells were donated by our collaborative group, which were cultivated with Dulbecco's modified Eagle's medium (DMEM) medium, containing 10% fetal calf serum (FBS) in incubator- CO_2 (5% CO_2 and 37°C atmospheres, Thermo Scientific). After standard operation, A549 cells with 60 ~ 70% cell fusion degree, were used in the electrochemical detection of H_2O_2 release from living cell in phosphate buffer (PBS, pH = 7.0).

3. Results And Discussion

3.1. Optimization of GO/ Cu_2O /GO electrode

Considering the size effect of Cu_2O nanocubes used in experiment, Cu_2O nanocubes ranging from 20 nm to 400 nm, were synthesized via seed-mediated approach [32] for our electrochemical measurement. As shown in Fig. 1A, Cu_2O single-layer electrode was used to evaluate size effect of Cu_2O nanocubes by cyclic voltammetry (CV) measurements in a range from -0.6 to +0.6 V at a scan rate of 100 mV/s in 0.1 M phosphate buffer (PBS, pH = 7.0). Apparently, 30 nm Cu_2O nanocubes revealed the strongest amperometric current, which were therefore chosen and used in the construction of sandwich-like structure and subsequent electrochemical experiments. The thickness of Cu_2O and GO layers in sandwich-like structure is also strongly related with the electrochemical activities of proposed sandwich-like GO/ Cu_2O /GO sensor. Obviously, the thickness of the layer will increase with the amount of the Cu_2O and GO used on ITO substrate, and the resulted response current in CV measurements varies accordingly. Cu_2O nanocubes ranging from 0.1 to 0.8 mg were used to compare the electrochemical activities of Cu_2O single-layer. As shown in Fig. 1B, the single-layer with 0.6 mg Cu_2O nanocubes revealed the highest peak current. Therefore, the optimal amount of Cu_2O used to fabricate sandwiched Cu_2O layer is 0.6 mg. Similarly, the thickness of GO layers was also studied by the related electrochemical performance in Fig. 1C. Different amounts (0.01, 0.02, 0.05, 0.1 and 0.2 mg) of GO were applied to find the highest amperometric response in CV measurements. The GO single-layer with 0.1 mg GO exhibited the maximum amperometric current. Based on these optimizations, 0.1 mg GO and 0.6 mg Cu_2O were applied to fabricate sandwich-like GO/ Cu_2O /GO sensor on ITO substrate in our further studies.

3.2 Characterization of the GO/ Cu_2O /GO electrode

Sequentially covering GO, Cu_2O and GO layers on ITO substrate, a 400 nm-thick sandwich-like GO/ Cu_2O /GO electrode is constructed to study the amperometric response in H_2O_2 detection. The cross-sectional structure of sandwich-like GO/ Cu_2O /GO electrode is presented by SEM characterization in

Fig. 2A. $\text{Cu}_2\text{O}/\text{GO}$ double-layer and Cu_2O single-layer on ITO substrate are also observed in Fig. 2B and Fig. 2C, respectively. About 300 nm-thick $\text{Cu}_2\text{O}/\text{GO}$ double-layer and 200 nm-thick Cu_2O single-layer were presented for controlled experiment. Especially in Fig. 2A, the surface of $\text{GO}/\text{Cu}_2\text{O}/\text{GO}$ lamellar structure becomes rough and some granules could be seen after GO layer's covering, which means the successful construction of 400 nm-thick $\text{GO}/\text{Cu}_2\text{O}/\text{GO}$ electrode on ITO substrate. The TEM image in Fig. 2D shows the general morphology of the Cu_2O nanocubes, which are sandwiched between GO layers in subsequent construction of sandwich-like structure. The Cu_2O nanocubes with ~ 30 nm in length show the relatively uniform distribution. Through the observation of HRTEM image of the inset in Fig. 2D, a lattice spacing of 0.24 nm, which corresponds to fringes of (111) planes of cubic-phase Cu_2O , is clearly demonstrated.

XRD measurements of Cu_2O , $\text{Cu}_2\text{O}/\text{GO}$ and $\text{GO}/\text{Cu}_2\text{O}/\text{GO}$ electrode are clearly observed in Fig. 2E. All the characteristic strong diffraction signals at 36.1° , 42.0° and 61.1° , can be indexed to the (111), (200) and (220) planes of cubic Cu_2O crystal phase, respectively. Moreover, the broad peak around 22° could be designated to the peaks of GO.

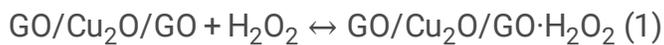
3.3. Electrochemical performances of $\text{GO}/\text{Cu}_2\text{O}/\text{GO}$ electrode

Cyclic voltammetry (CV) is a method for obtaining qualitative information of various electrochemical reactions [7, 36]. To investigate the newly developed sandwich-like structure, CVs were employed in a range from -0.6 to $+0.6$ V at a scan rate of 100 mV/s in 0.1 M phosphate buffer (PBS, pH = 7.0) on GO, Cu_2O , $\text{Cu}_2\text{O}/\text{GO}$ and $\text{GO}/\text{Cu}_2\text{O}/\text{GO}$ on ITO substrate, respectively.

As shown in Fig. 3A, the GO single-layer exhibits no obvious response at continuous scanning, while the Cu_2O single-layer displays a couple of redox peaks around -0.3 and 0.1 V assigned to the $\text{Cu}^+/\text{Cu}^{2+}$ redox process. After the formation of $\text{Cu}_2\text{O}/\text{GO}$ double-layers, the surface area of electrode has become enlarged compared to the bare GO single-layer according to the introduction of more active sites by Cu_2O nanocubes, which is conducive to increasing the response peak current. In addition, a new couple of redox cycle about -0.4 and 0.2 V further illuminated the combination between Cu_2O and GO layers, and clearly exhibited the improved redox ability of the as-prepared $\text{Cu}_2\text{O}/\text{GO}$ double-layer, which was in accordance with the reported results [37]. Furthermore, inspired by better performance of sandwich-like structure on graphene-based materials [31], GO layer was therefore constructed on the top of $\text{Cu}_2\text{O}/\text{GO}$ layers to form sandwich-like $\text{GO}/\text{Cu}_2\text{O}/\text{GO}$ structure on ITO substrate. The corresponding response current of the $\text{GO}/\text{Cu}_2\text{O}/\text{GO}/\text{ITO}$ was notably increased in Fig. 3A, which provided primary evidence that the prepared sandwich-like $\text{GO}/\text{Cu}_2\text{O}/\text{GO}$ electrode revealed superb electrocatalytic activity.

3.4. Amperometric response and calibration curve for H_2O_2 detection

According to previous reports about the mechanism of H_2O_2 [38], the corresponding mechanism on resultant GO/Cu₂O/GO was deduced as follows:



H_2O_2 was firstly absorbed on the surface of sandwich-like GO/Cu₂O/GO structures to form GO/Cu₂O/GO·H₂O₂. Then GO/Cu₂O/GO·H₂O₂ led to the formation of two OH⁻ ions after successively receiving two electrons. Simultaneously, GO/Cu₂O/GO·H₂O₂ reverted to GO/Cu₂O/GO. In the light of the mechanism, it could be deduced that the adsorption process and electron transfer process are the two main limitations to control the rate of H_2O_2 reduction, GO sheets were applied with Cu₂O nanocubes to construct the sandwich-like structure for the H_2O_2 detection. Figure 3B shows the CVs of sandwich-like GO/Cu₂O/GO electrode in the presence of H_2O_2 at different concentrations from 0 to 5 mM. When the concentration of H_2O_2 increases and the oxidation peak current decreases, the reduction peak current at about -0.3 V in CVs is significantly enhanced, which suggests that H_2O_2 can easily be reduced by modified sandwich-like electrode in a wide range of concentrations.

The sensitivity of fabricated sandwich-like GO/Cu₂O/GO sensors for H_2O_2 detection can be further evaluated by chronoamperometry. Figure 3C exhibited the amperometric *i* - *t* response of GO/Cu₂O/GO sensors to the successive H_2O_2 injection into 0.1 M deoxygenated PBS (pH = 7.0) at -0.3 V vs Ag/AgCl with continuously stirring. Obviously, the GO/Cu₂O/GO electrode responded quickly, and the response current attained a stable state within a few seconds, which indicated the high electron transfer efficiency and fast diffusion of H_2O_2 on the electrode surface. As displayed in Fig. 3D, the reduction peak current (*I*) was directly proportional to the concentration (*C*) of H_2O_2 , which showed two different kinds of linear relationship of $I (\mu\text{A}) = 0.054 + 75.88C (\text{mM})$ ($R^2 = 0.995$) in the range of 0.25 μM - 6 mM and $I (\mu\text{A}) = 4.099 + 13.041C (\text{mM})$ ($R^2 = 0.996$) in the range of 6 - 20 mM (Fig. S1). At different H_2O_2 concentrations, the different linear relationships may be caused by different electrocatalytic reduction kinetics. H_2O_2 reduction is dominated by surface adsorption of H_2O_2 at low concentration, whereas the surface diffusion of H_2O_2 on electrode changes to be the dominant step at high concentration, which in turn results in a decreased linear relationship slope [39]. In addition, based on a signal-to-noise factor of 3 (S/N = 3), the limit of detection (LOD) for the H_2O_2 sensor can be calculated to be 0.2 μM.

Table 1

Comparison of detection limit and linear range to Cu₂O-based H₂O₂ biosensors.

| Electrode | Linear range (μM) | Detection limit (μM) | Ref. |
|-----------------------------------|-------------------|----------------------|-----------|
| 2D Cu ₂ O-rGO-P | 5-10560 | 3.78 | [16] |
| Graphene-Cu ₂ O | 300–3300 | 3.3 | [27] |
| Porous Cu ₂ O | 5-1500 | 1.5 | [40] |
| Hierarchical Cu ₂ O | < 22 | 0.039 | [41] |
| Cu ₂ O Nanowire Arrays | 50-20000 | 10 | [42] |
| Ag-Au/Cu ₂ O | 0-1400 | 1.3 | [39] |
| GO/Cu ₂ O/GO | 0.25–20000 | 0.1 | This work |

A comparison of linear range and LOD of GO/Cu₂O/GO/ITO electrode with other Cu₂O-based H₂O₂ sensors are listed in Table 1, indicating that electrochemical performances of our sandwich-like GO/Cu₂O/GO sensors are preferred than those obtained and reported recently. Thus, the sandwich-like electrodes can be used to accurately and sensitively monitor H₂O₂ due to its outstanding design concept and simple construction.

3.5. Stability and selectivity of GO/Cu₂O/GO electrode

As shown in Fig. S2, the stabilities of Cu₂O, Cu₂O/GO, and GO/Cu₂O/GO electrodes were evaluated in PBS (pH = 7.0) during the successive addition of H₂O₂ for 8 cycles. The response current of Cu₂O and Cu₂O/GO layers on ITO substrate decreased during cyclic experiments in Fig. S2A and Fig. S2B, due to the instability of Cu₂O itself. Interestingly, as the formation of sandwich-like structures, the outer GO layer revealed the improvement of the stability in H₂O₂ detection after initial activation of GO/Cu₂O/GO sensors shown in Fig. S2C.

Species that can be oxidized easily, such as ascorbic acid (AA), uric acid (UA) and proteins, usually coexist with H₂O₂ in vivo. Therefore, interference effect is another important parameter affecting the electrochemical response except stability. Herein, the electrochemical sensitivity of the above interfering species was also investigated. The anti-interference effect of sandwich-like GO/Cu₂O/GO sensors was tested by successive addition of 0.1 mM H₂O₂ at -0.30 V vs Ag/AgCl, followed by the addition of 1 mM glucose, 1 mM AA, 1 mM glycine (Gly), 1 mM histidine (His) and 0.1 mM H₂O₂ in a 0.1 M PBS solution, as shown in Fig. 4A. It was clearly observed that insignificant current responses have been recorded for these interfering species compared to that from H₂O₂ addition. The low potential (-0.3 V) applied for detection and ingenious design of the sandwich-like electrode endowed the sensor with high selectivity

and stability. The observations further suggested that special multilamellar GO/Cu₂O/GO sensors demonstrated pleasant specificities for H₂O₂ detection.

3.6. Measurements of H₂O₂ release from living cells

High sensitivity and low detection limit being achieved, the sandwich-like GO/Cu₂O/GO sensor can be used to detect H₂O₂ release from living cells. The amperometric current response flux of H₂O₂ with about 5.0×10^6 A549 cells in 5 mL of physiological PBS buffer was recorded at -0.3 V (vs Ag/AgCl) under 37 degrees. Ascorbic acid (AA) can motivate cell generation of large amount of H₂O₂, which can be chosen as the stimulus [43, 44]. Figure 4B revealed the amperometric response of GO/Cu₂O/GO sensor in the presence of A549 cells triggered by AA in PBS buffer solution (pH = 7). As a control, experiments without A549 cells (black line, Fig. 4B) or the cells with catalase (red line, Fig. 4B) were measured and revealed no response of current when adding certain amount of AA solution. Apparently, a significant peak current response of H₂O₂ flux with the A549 cells, was recorded with successive addition of the stimulus, i.e. AA. However, the peak current declined ca. 58.3% after 6 s because the H₂O₂ of cellular efflux re-entered into cells [44]. The release rates of H₂O₂ are dependent on the concentration of AA, which could be estimated by the slopes of each injection. The maximum current change of ~ 0.111 μ A upon injection of 2 μ M AA roughly corresponds to H₂O₂ concentration of 0.0751 μ M in cell solution according to the linear relationship in Fig. 3D. Considering the total amount of 5 mL cell solution, ~ 0.37 nmol H₂O₂ were released from the A549 cell. The number of the cell used in the measurement is 5.0×10^6 , which indicates that H₂O₂ released from each cell is about 75 amol. These results demonstrated that the proposed sandwich-like GO/Cu₂O/GO electrode presented a sensitive probe for extracellular H₂O₂ detection and could be exploited for physiological and pathological studies.

4. Conclusion

In summary, a well-constructed and easy-established sandwich-like GO/Cu₂O/GO sensor has been synthesized for the determination of H₂O₂ molecule. The special multilamellar GO/Cu₂O/GO electrode enhanced the peak current, high stability and sensibility in electrochemical measurements. The lower LOD value (0.2 μ M) and broader response range (0.25–20000 μ M) of sandwich-like GO/Cu₂O/GO sensor for H₂O₂ detection are superior to that from the reported Cu₂O-based biosensors. Furthermore, the lamellar structured sensor has been successfully employed to detect H₂O₂ in the living A549 cells with satisfactory results. Therefore, easy construction, low cost and high stability of the sandwich-like structure reveal the potential application of this novel structure in H₂O₂ detection for monitoring intracellular signaling transduction and normal cell functions.

Supplementary Information

Additional file 1: Fig. S1. Calibration curve of amperometric response to H_2O_2 detection in the concentration range of 6 – 20 mM. Fig. S2. Amperometric performance of H_2O_2 detection in Cu_2O single-layer (A), Cu_2O /GO double-layer (B) and sandwich-like GO/ Cu_2O /GO electrodes.

Abbreviations

SEM: Scanning electron microscopy; TEM: Transmission electron microscopy; XRD: X-ray diffraction; HRTEM: High-resolution transmission electron microscopy; LOD: low limit of detection; CV: Cyclic voltammetry; NPs: Nanoparticles; 2D: Two-dimensional; GO: Graphene oxide; ITO: Indium tin oxide; DMEM: Dulbecco's modified Eagle's medium; FBS: Fetal calf serum; PBS: Phosphate buffer; AA: Ascorbic acid; UA: uric acid; Gly: Glycine; His: Histidine

Declarations

Acknowledgements

Not applicable.

Authors' contributions

The manuscript was written through contributions of all authors. DHY designed, performed the experiments. JYT analyzed the experiment data and wrote the manuscript. RXM assisted with interpretation of the data from electrochemical test. FW, XZJ and CLL helped to check the manuscript before submission. CLL conceived of the study and all authors read and approved the final version of the manuscript.

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Availability of Data and Materials

All data and materials are fully available without restriction.

Competing Interests

The authors declare that they have no competing interests.

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Figures

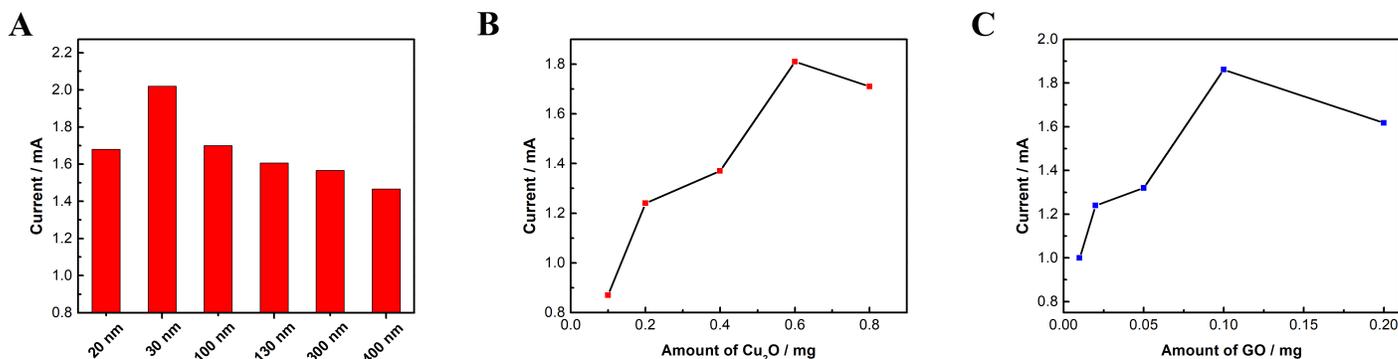


Figure 1

Size effect of Cu_2O nanocubes (A) and optimization of the amount of Cu_2O (B) and GO (C) in their single-layer electrode by cyclic voltammetry measurements at a scan rate of 100 mV/s in 0.1 M PBS (pH=7.0) vs Ag/AgCl.

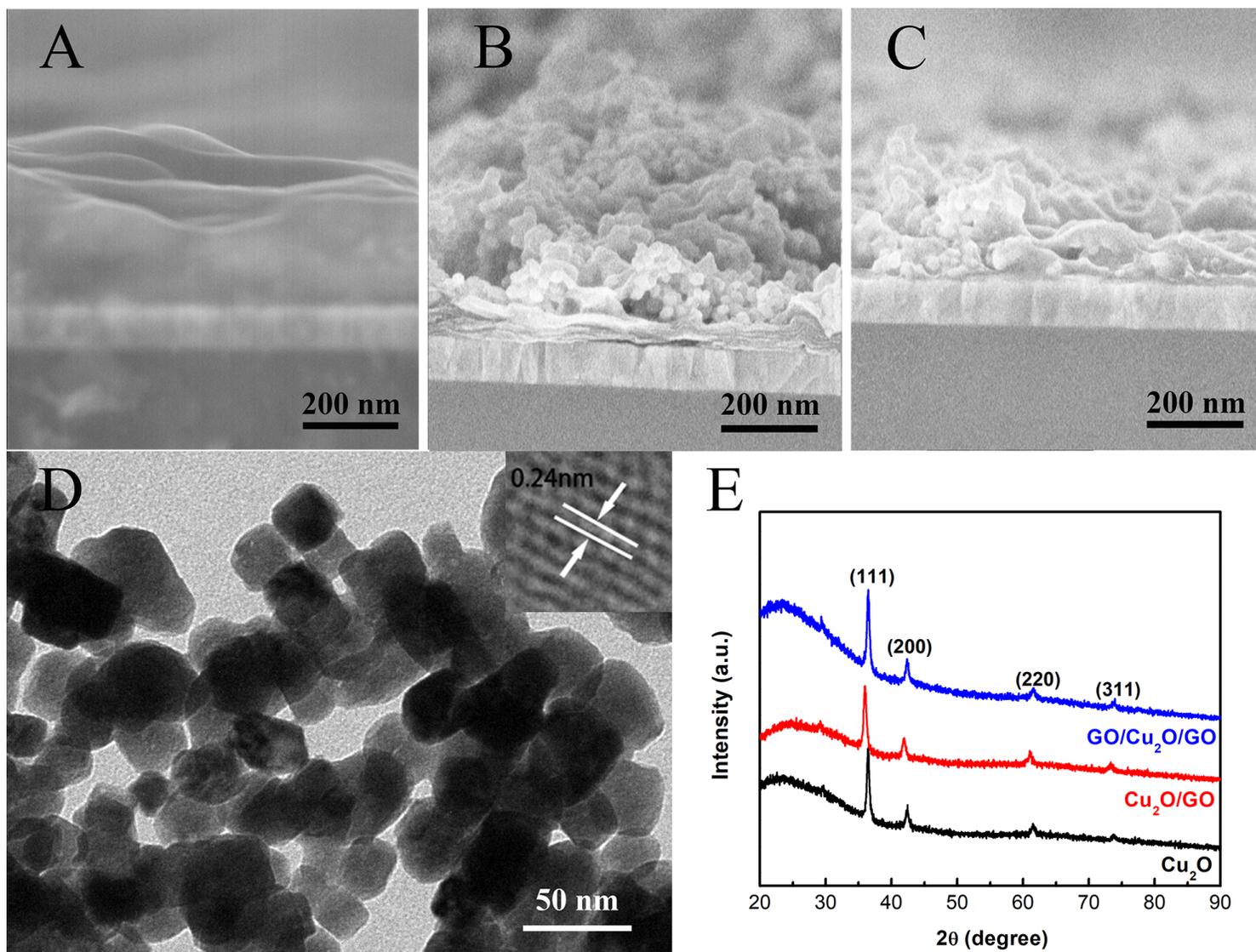


Figure 2

SEM images of cross-sectional GO/Cu₂O/GO/ITO (A), Cu₂O/GO/ITO (B) and Cu₂O/ITO (C) electrode. TEM image of sandwiched Cu₂O nanocubes (D) and XRD pattern (E) of GO/Cu₂O/GO/ITO, Cu₂O/GO/ITO and Cu₂O/ITO. Inset in (D): the lattice fringe of Cu₂O nanocubes.

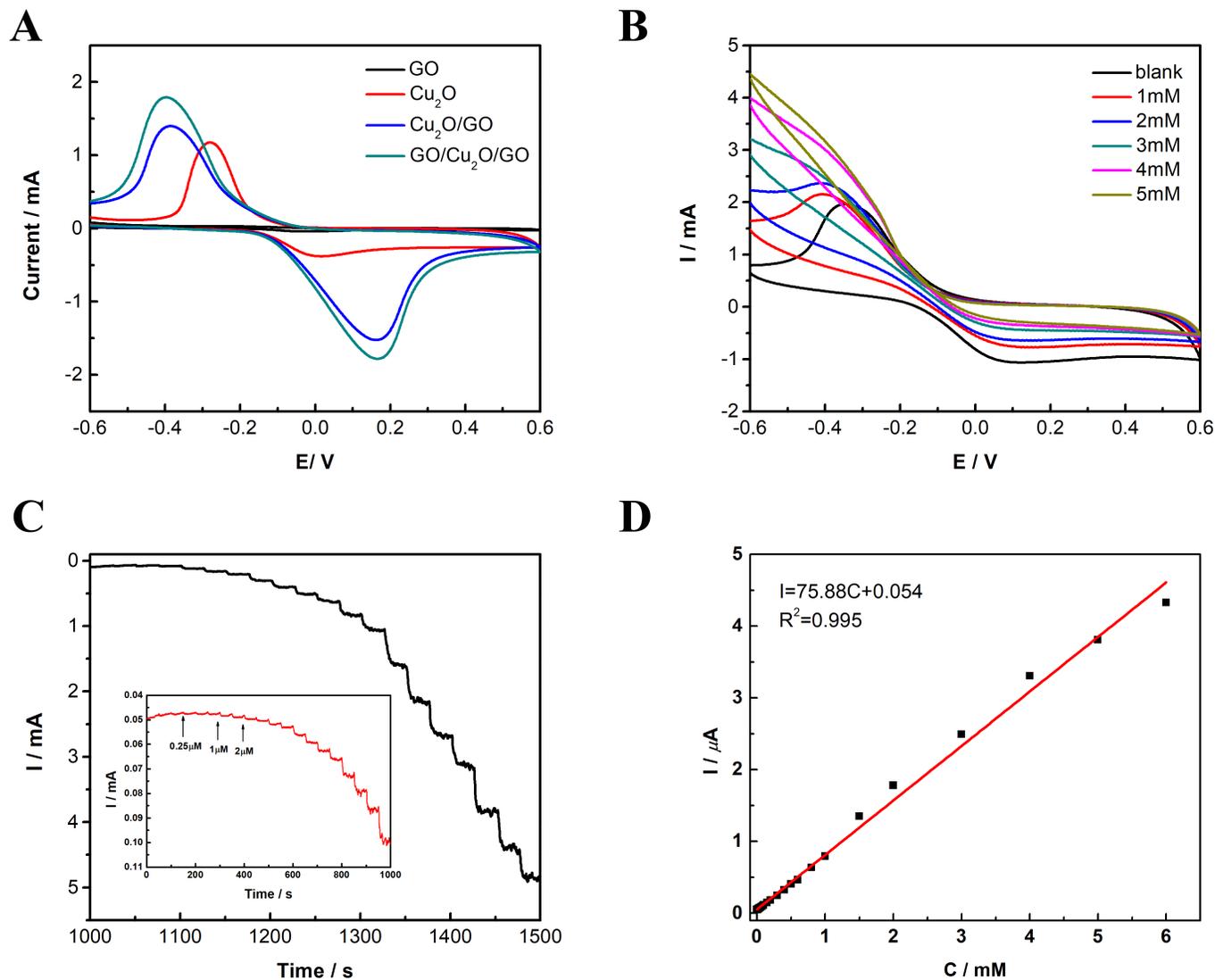


Figure 3

CV curves (A) of Cu₂O/ITO, GO/ITO, Cu₂O/GO/ITO and GO/Cu₂O/GO /ITO electrode and concentration effect (B) of H₂O₂ by GO/Cu₂O/GO electrode in CV measurements. Amperometric response (C) of GO/Cu₂O/GO to the successive adding H₂O₂ in a range of 0.25 μM -20 mM and the corresponding calibration curve (D) of GO/Cu₂O/GO sensor for H₂O₂ detection. All the electrochemical measurements are taken at a scan rate of 100 mV/s in 0.1 M PBS (pH=7.0) vs Ag/AgCl electrode.

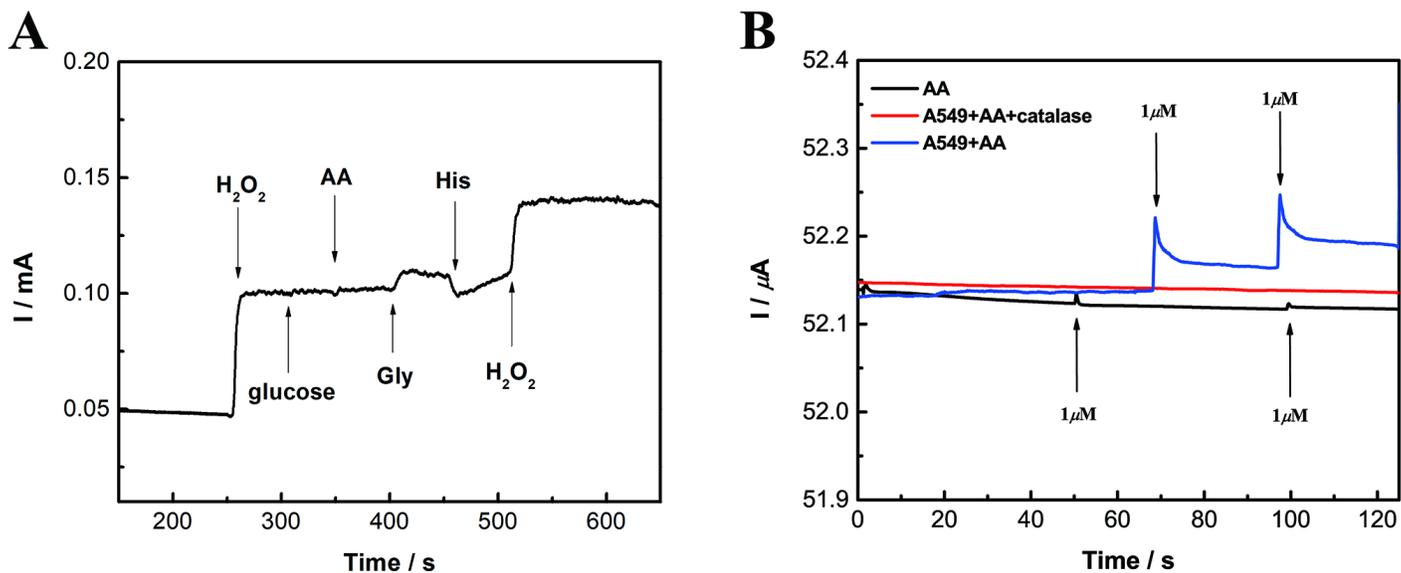


Figure 4

Amperometric response of sandwich-like GO/Cu₂O/GO sensor to anti-interference measurements (A), and to A549 living cells (B) stimulated by AA in PBS (pH = 7.0)

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