Graphene Quantum Dots/Gold Electrode and Its Application in Living Cells H_2O_2 Detection

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Methods

Materials

Graphite (crystalline powders, ~ 500 meshes) was purchased from Shanghai Yifan Graphite Co., Ltd., Shanghai, China. 3，3’，5，5’-Tetramethylbenzidine (TMB), KCl, KH_2PO_4, K_2HPO_4, NaAc, HAc, and 30% H_2O_2 were obtained from Sinopharm Chemical Reagent Co., Ltd., Shanghai, China. Concentrated H_2SO_4 was acquired from Shanghai Lingfeng Chemical Reagent Co., Ltd., Shanghai, China. Cysteamine was acquired from Aladdin Chemistry Co., Ltd., Shanghai, China. N-(3-Dimethylaminopropy)-N’–ethylcarbodiimide hydrochloride (EDC) were obtained from Sigma-aldrich Co., Ltd. N-Hydroxysuccinimide (NHS) was purchased from Thermo Scientific Co., Ltd. Phorbol-12-myristate-13-acetate (PMA) was purchased from Beyotime Institute of Biotechnology Co., Ltd., Shanghai, China. All of the reagents were used as received.

1.1 Preparation of graphene quantum dots

GO and GQDs were generated as described in our previous work.[26] In a typical experiment, 20 mL, 1 mg/mL GO aqueous suspension and 1 mL, 30% H_2O_2 were mixed in a quartz tube under vigorous stirring. The reaction was initiated by exposing the quartz tube to a mercury lamp (365 nm, 1000 W). The reaction products were dialyzed in ultra-pure water for two days to remove the trace H_2O_2, and other small molecular reaction products.

1.2 Instrumentations

Atomic Force Microscope (AFM) images were acquired using a Multimode Nanoscope V scanning probe microscopy system (Bruker, USA). The specimen was prepared by casting the aqueous suspensions of GQDs on freshly cleaved mica surface and dried in air. Scanning electron microscopic (SEM) images of modified GC electrode were acquired on an Ultra 55 field emission scanning electron microscopy (Zeiss, Germany) with 5 kV of acceleration voltage. UV-visible absorption spectra were recorded on a Shimadzu UV-2550 (Shimadzu, Japan).

1.3 Peroxidase activity assay

The peroxidase activity assays were carried out by monitoring the absorbance change at 652 nm for 5 min on UV-visible spectrophotometer. In a typical experiment, 40 μg GQDs in 1 mL NaAc buffer solution (0.1 M, pH 3.5) were mixed with 0.9 mM TMB and 50 mM H_2O_2.

1.4 Modification of Au electrode by GQDs

To modify the Au electrode, the cysteamine monolayer was assembled on the Au electrode’s surface first. The bare Au electrode was polished to a mirrorlike surface with 1.0, 0.3, and 0.05 μm α-alumina and immersed in piranha solution (a hot solution of 1:3 vol/ vol 30% H_2O_2/conc. H_2SO_4) for 1 min. The Au substrate was then subjected to mass water washing and ultrasonical cleaning using water and methanol successively. The cysteamine monolayer was prepared by dipping the clean Au electrode upright into 0.05 M cysteamine solutions for 4 h at 25 ℃. Then the electrode was rinsed with water and dried with a stream of N_2. At the same time, 0.4 mg GQDs were dispersed in 1 mL water with 3.6 mg EDC and 1.0 mg NHS to convert the carboxyl groups into active carbodiimide esters. The cysteamine modified gold electrode was dipped upright into the GQDs solution for 12 h and then rinsing with water and drying in a stream of N_2.

1.5 Electrochemical measurements

The electrochemical measurements were performed on a CHI 660C electrochemical workstation (Chen Hua Co., China) using a three electrode system, with a bare Au or the modified Au electrode as a working electrode, platinum wire as a counter electrode, and a saturated calomel electrode as reference electrode. The electrolyte solution purged with pure N_2 for 15 min to remove oxygen before the electrochemical measurement.

1.6 Detection of H_2O_2 in living cells

Human breast cancer MCF-7 cells were obtained from Cell Bank (Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences). The cells were maintained in a culture medium consisting of RPMI-1640 medium supplemented with 10% FBS and 100 U mL^{-1} penicillin-streptomycin (37 ℃, 5% CO_2). The MCF-7 cells were centrifuged to obtain a cell-packed pellet (~ 1.3×10^6 cells) for the electrochemical experiments.

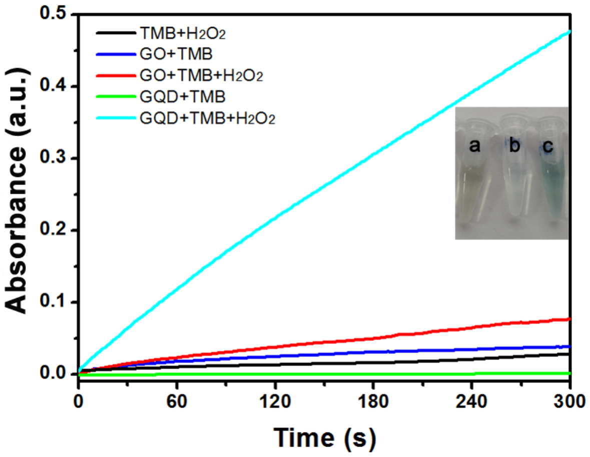

Figure S1. The time-dependent absorbance changes at 652 nm in different reaction systems. Inset was the typical photographs of different solutions, (a) GQDs + TMB; (b) TMB+ H_2O_2; (c) GQDs +TMB+ H_2O_2 in 0.1 M, pH 3.5 NaAc buffer.

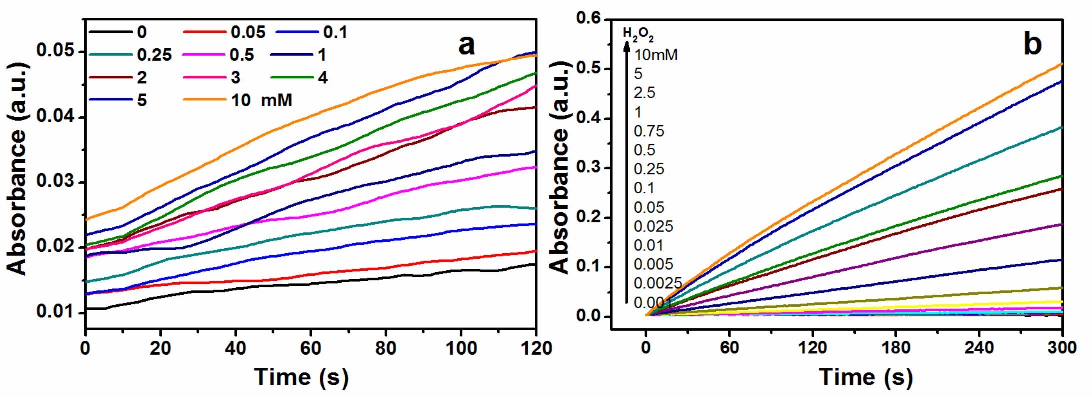

Figure S2. The time-dependent absorbance changes of the reaction mixture of GO +TMB+H_2O_2 (a) and GQDs +TMB+H_2O_2(b) at 652 nm under varying concentrations of H_2O_2 using 40 μg GO and 40 μg GQDs as catalysts.

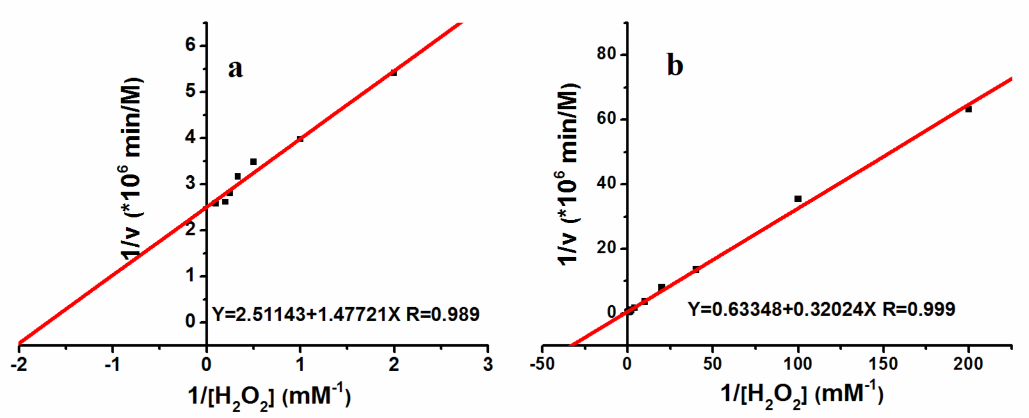

Figure S3. The double reciprocal plots of the initial reaction rates of TMB + H_2O_2 with GO (a) and GQDs (b) versus H_2O_2 concentration.

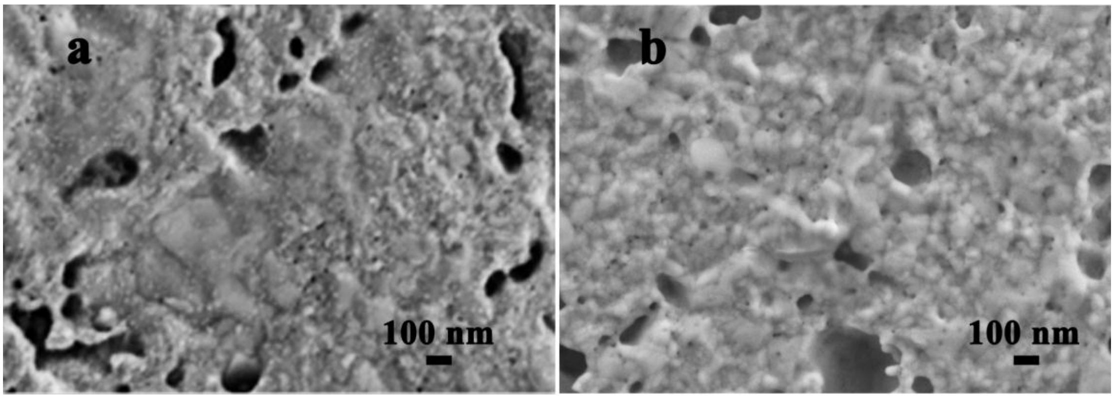

Figure S4. SEM images of the GQDs/Au (a) and GO/Au (b) electrodes that stored for more than 30 days at room temperature (~20 ℃)

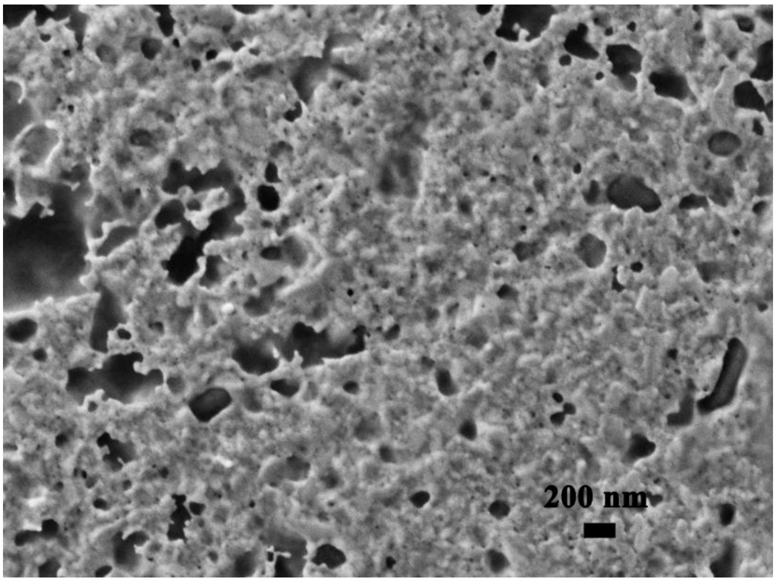

Figure S5. SEM image of the GQDs modified Au electrode through casting method.

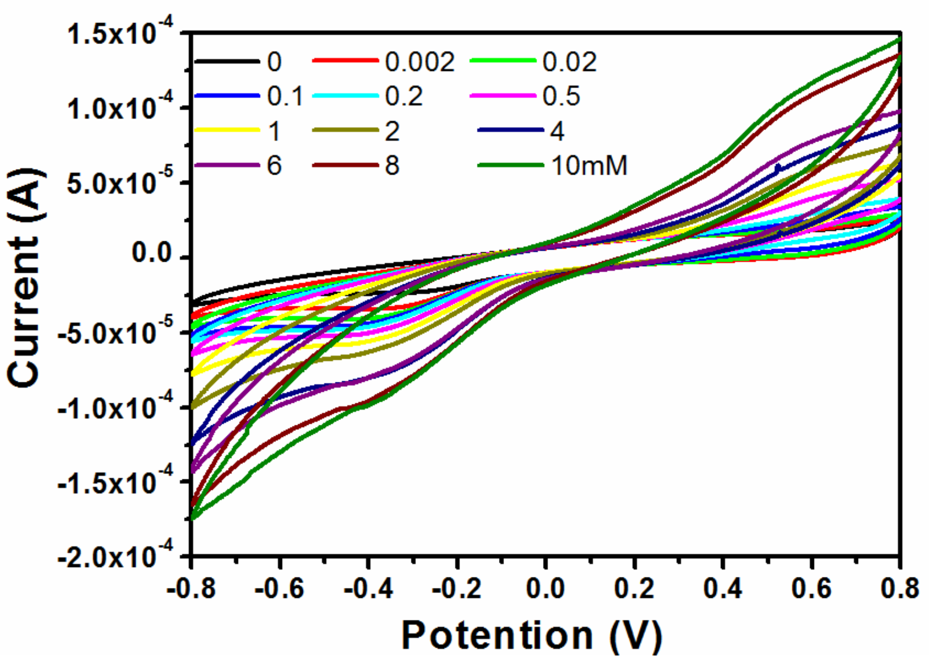

Figure S6. CVs of the GQDs/Au electrode in phosphate buffer (0.01 M, pH 7) with successive addition of H_2O_2 at 50 mV/s.

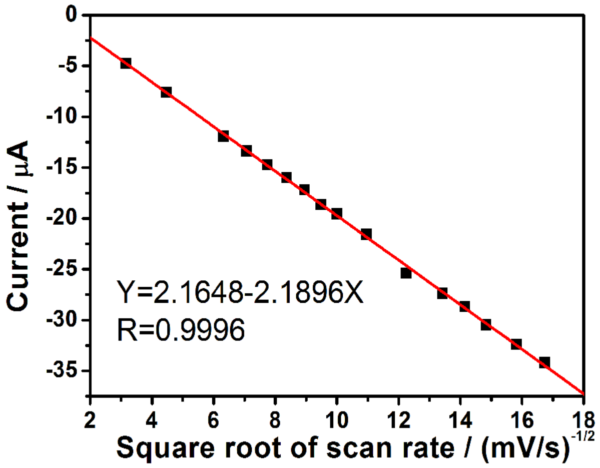

Figure S7. The peak current intensity, which was obtained from cyclic voltammograms of the GQDs/Au electrodes in PBS (0.01 M, pH 7) buffer at different scan rates increases linearly with the square root of scan rate.

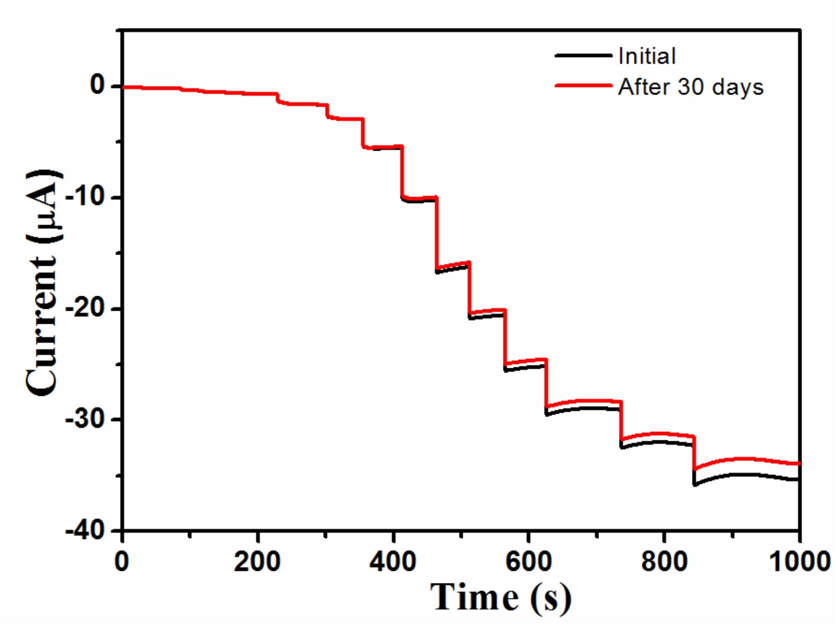

Figure S8. Comparison of the amperometric responses of the GQDs/Au electrodes that fresh prepared and stored 30 days to successive addition of H2O2 at -0.4 V vs. SCE in PBS (0.01 M, pH 7) buffer.

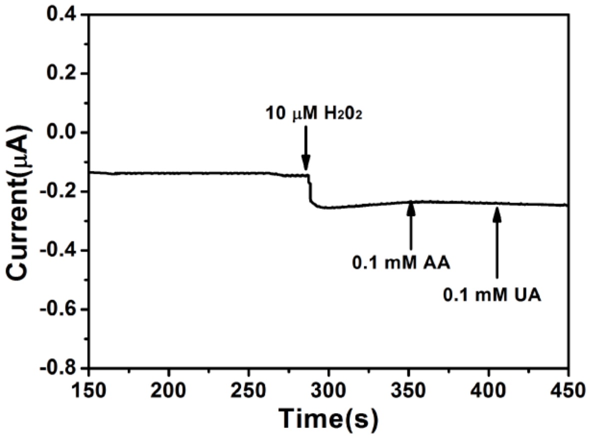

Figure S9. Amperometric responses of the GQDs/Au electrode to the addition of H_2O_2, L-ascorbic acid (AA) and uric acid (UA) at an applied potential of - 0.4 V.

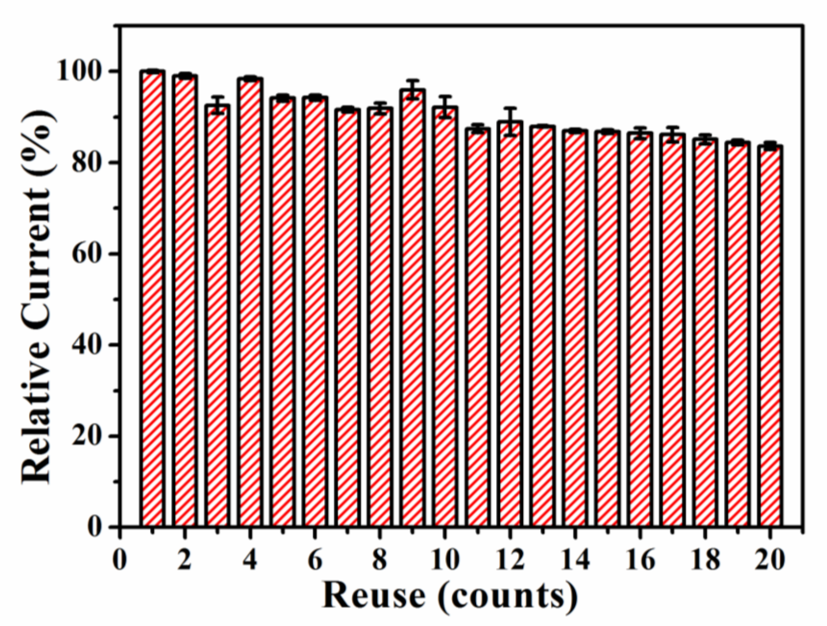

Figure S10. Reuse of the GQDs/Au electrode in PBS (0.01 M, pH 7).