Supporting Information

Electron Spin Resonance and Fluorescence Imaging Assisted Electrochemical Approach for Accurate and Comprehensive Monitoring Cellular Hydrogen Peroxide Dynamics

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1. Preparation of F-C-GO and AuNPs

Graphene oxide (GO) was prepared by a modified Hummers' method[1] and used as the starting material for preparing the phenylalanine (F) and cysteine (C) functionalized GO (F-C-GO). In a typical synthesis of F-C-GO, 0.004 g of GO was dispersed in 10-mL ultrapure water and ultrasonicated for 60 min to obtain homogeneous solution. Then, 10 mg of 1-Ethyl-3-(3-dimethylamin-opropyl) carbodiimide hydrochloride (EDC) and 3 mg of N-hydroxysuccinimide (NHS) were added into the dispersed GO solution to prepare the amine reactive GO-bearing-NHS ester. After stirring for 2.5 h and washing with ultrapure water for three times, the resultant was mixed with L-phenylalanine (1 mg mL^{-1}) and L-cysteine (1 mg mL^{-1}) and stirred at room temperature for 12 h to yield F-C-GO. The obtained F-C-GO could be well re-dispersed in ultrapure water. AuNPs were prepared following a previously reported method[2]. Briefly, all glassware used in the preparation was thoroughly cleaned in aqua regia which was prepared by mixing concentrated HCl (37%) and concentrated HNO_3 (65%) at a volume ratio of 3:1, rinsed in ultrapure water, and oven-dried prior to use. *Caution:* aqua regia is extremely dangerous and should be handled with extreme caution. Gloves and eye protection are required for handling. In a 250-mL round-bottom flask equipped with a condenser, 4.13 mL of 1% $HAuCl_4$ was added into 100-mL ultrapure water. After the $HAuCl_4$ solution was heated to boiling, 11.3 mL of 1% citrate was quickly added and then kept for about 30 min, resulting in a color changing from pale yellow to burgundy.

2. Contact angel, chemical composition, and morphology characterization

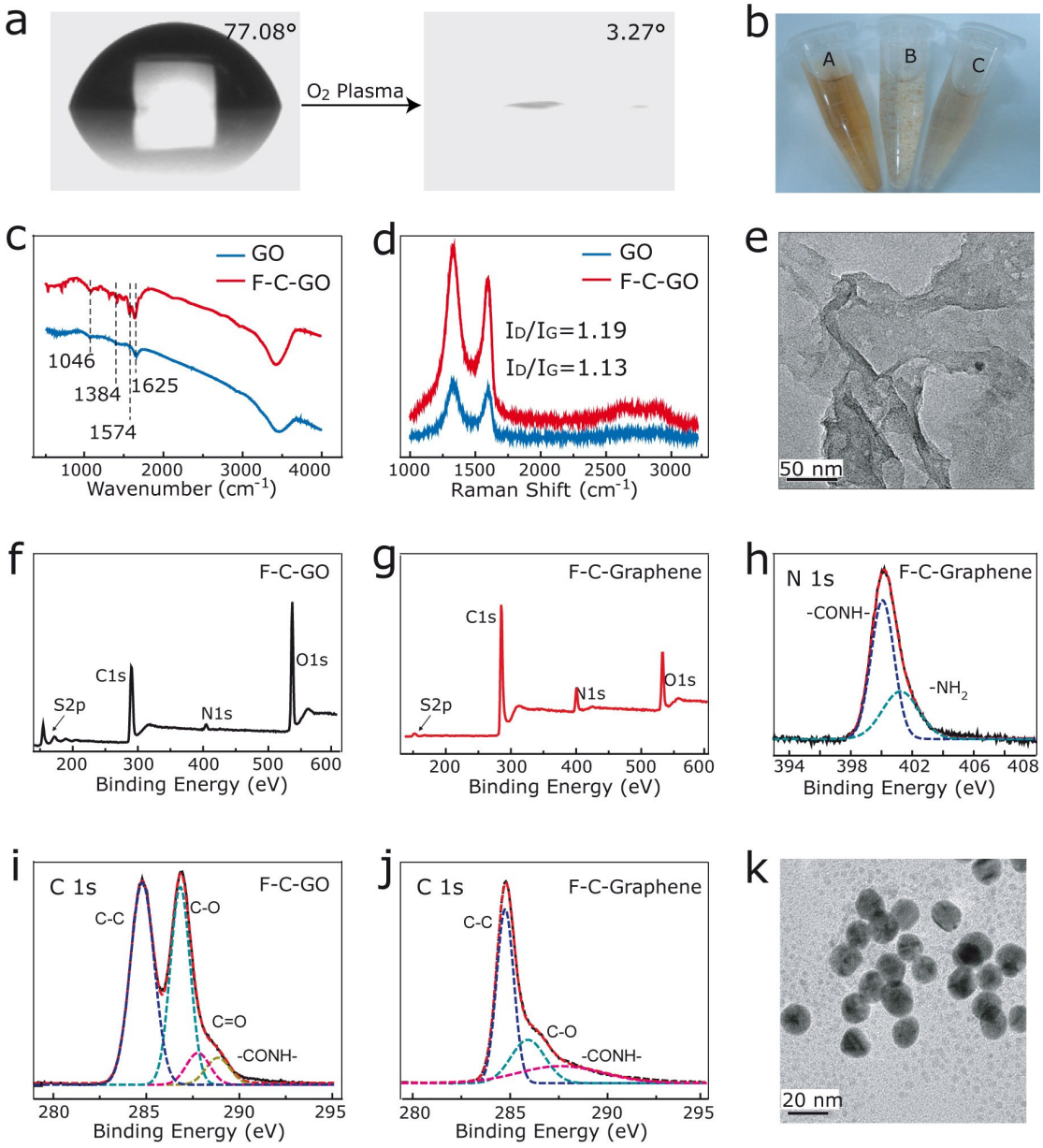

Fig. S1 (a) Contact angle images of ITO without (left) and with (right) oxygen plasma treatment. (b) Photograph of GO (A), GO-bearing-NHS ester (B), and F-C-GO (C). (c) FTIR spectra of GO and F-C-GO. (d) Raman spectra of GO and F-C-GO. (e) TEM image of F-C-Graphene. (f, g) XPS survey spectra of F-C-GO and F-C-Graphene. (h) High-resolution XPS N 1s spectrum of F-C-Grapene. (i, j) High-resolution XPS C 1s spectra of F-C-GO and F-C-Graphene. (k) TEM image of AuNPs.

Oxygen plasma treatment was used to improve the hydrophilic property of ITO, providing a good spreading effect for F-C-GO nanosheets. The contact angle results in Fig. S1a showed that two obviously different contact angles of ITO without (77.08°) and with (3.27°) oxygen plasma treatment, clearly indicating the improvement of hydrophilic property of ITO after oxygen plasma treatment.

In order to obtain a high-quality F-C-Graphene that played a key role in the electron transfer property of the working electrode, a well-dispersed F-C-GO was first prepared and characterized, following with a successful preparation of F-C-Graphene/ITO by the *in situ* reduction of F-C-GO on ITO under the hydrazine vapor at 60 ºC for 6 h. As shown in Fig. S1b, we can see that a well-dispersed aqueous solution of F-C-GO was successfully synthesized. FTIR and Raman spectroscopies were carried out to characterize the chemical composition of F-C-GO. A successful attachment of amino acid onto the GO through amidation was confirmed by monitoring the evolution of vibrational bands of the functional groups as shown in the FTIR spectra (Fig. S1c). In the FTIR spectra of GO, the peaks at 1384, 1046, and 1625 cm^{-1} were attributed to the deformation vibration of O-H, stretching vibration of epoxy C-O and aromatic C=C, respectively. After reaction with amino acids, a distinct peak at 1574 cm^{-1} attributed to the amide group appeared in the FTIR spectra of F-C-GO indicated the covalent amide linkage between GO and amino acids. Further evidence for the existence of amide groups in F-C-Graphene and the attachment of phenylalanine on the graphene nanosheets could be found by Raman spectroscopy (Fig. S1d). Raman spectroscopy is a useful nondestructive tool to characterize carbonaceous materials, particularly for studying ordered and disordered carbon structures. The well-known characteristics of carbon materials in Raman spectra are the G band which is usually assigned to the E_{2g} phonon of sp^2-hybridized carbon atoms and the D band which is a breathing mode of κ-point phonons of A_{1g} symmetry caused by local defects and disorders, particularly the defects located at the edges or in samples. The D bands at 1340 cm^{-1} indicated the typical disordered feature with high density of defects for GO and F-C-GO (Fig. S1d). Besides, we calculated the intensity ratio of D and G bands (I_D/I_G) for GO and F-C-GO. An increased I_D/I_G ratio from 1.13 to 1.19 was observed, suggesting increase of the disordered structure after chemical decoration of amino acids onto GO. This result was consistent with the FTIR spectrum and confirmed the successful synthesis of F-C-GO.

Fig. S1e shows the morphology of the synthesized F-C-Graphene. It can be seen the typical graphene thin flakes without agglomeration owing to the introduction of phenylalanine and cysteine as the stabilizer. The noncovalent modification of graphene sheets through π-π interactions with aromatic organic molecules such as phenylalanine and covalent functionalization both contributed to the aqueous

dispersions of graphene sheets.

X-ray photoemission spectroscopy (XPS) was carried out to characterize the chemical composition of F-C-Graphene. The appearance of N 1s and S 2p peaks in the XPS survey spectrum for F-C-Graphene indicated the attachment of amino acids on the graphene sheets (Fig. S1f,g). Furthermore, the C 1s and N 1s XPS spectra of F-C-Graphene in Fig. S1h,j showed the presence of amine NH_2 (286.8 eV in C 1s and 401.5 eV in N 1s) and amide bonds N-C=O (288.8 eV in C 1s and 400.1 eV in N 1s), suggesting the amino acids had been covalently linked to the GO surface via formation of amide bonds and remained in F-C-Graphene after reduction. As expected, the C 1s spectrum of F-C-GO given in Fig. S1i displayed pronounced peaks at 286.8 and 287.8 eV, which were attributed to C-O and C=O bonds, respectively. In contrast, the peak intensities of C-O and C=O significantly decreased as shown in the C 1s spectrum of F-C-Graphene (Fig. S1j). Especially, the peak of C=O almost vanished, indicating a well reduction of F-C-GO via hydrazine steam. The atomic percentage of oxygen in F-C-GO was estimated to be 30.3% before reduction, and decreased to be 13.2% after reduction. These results implied that the deoxygenation of F-C-GO by *in situ* reduction with hydrazine vapor obviously re-established the conjugated graphene network in F-C-Graphene. Fig. S1k shows that AuNPs with an average size of about 10 nm were uniformly distributed.

3. Optimization of the concentration of F-C-GO for modification

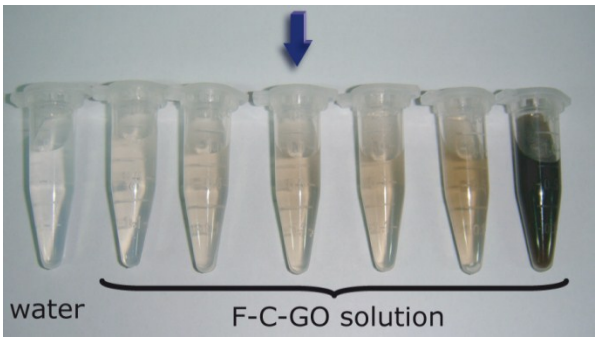

Fig. S2 Photograph of water and different concentrations of F-C-GO as 0.02, 0.04, 0.06, 0.08, 0.12, and 1mg mL^{-1} from left to right.

Fig. S2 is the photograph of F-C-GO solution with concentrations of 0.02, 0.04, 0.06, 0.08, 0.12 mg mL^{-1}, which clearly showed the gradient color change of the solution from pale yellow to dark brown. In our experiment, the working electrode

modified with 0.06 mg mL^{-1} of F-C-GO (as indicated by the purple arrow) displayed the largest redox current, indicating its high sensitivity for electrochemical detection, and thus was used for the preparation of our working electrode..

4. The selectivity of the electrode towards other biological relevant ROS and redox compounds

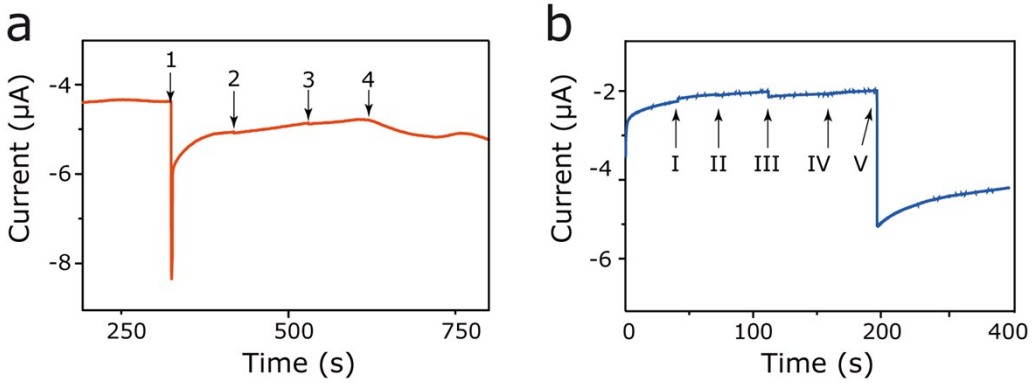

Fig. S3. (a) The amperometric response of the working electrode (RGDFC/HRP/AuNP/ F-C-Graphene/ITO) to the addition of (1) 1 mM H_2O_2, (2) 1 mM hydroxyl radical, (3) 1mM peroxynitrite, and (4) 1 mM hypochlorous acid, respectively. Hydroxyl radical was generated by the Fenton reaction (the molar ratio of H_2O_2/Fe^{2+} is 5:1). And peroxynitrite was chemically provided by mixing H_2O_2 with $NaNO_2$ (the molar ratio of $H_2O_2/NaNO_2$ is 1:1). (b) The amperometric responses of the working electrode (RGDFC/HRP/AuNP/ F-C-Graphene/ITO) to addition of 1 mM (I) ascorbic acid, (II) glutamic acid, (III) glutathione, (IV) cysteine, and (V) 0.5 mM H_2O_2, respectively. Three independent experiments were performed in triplicate.

5. Study of cell adhesion and proliferation on different modified electrodes

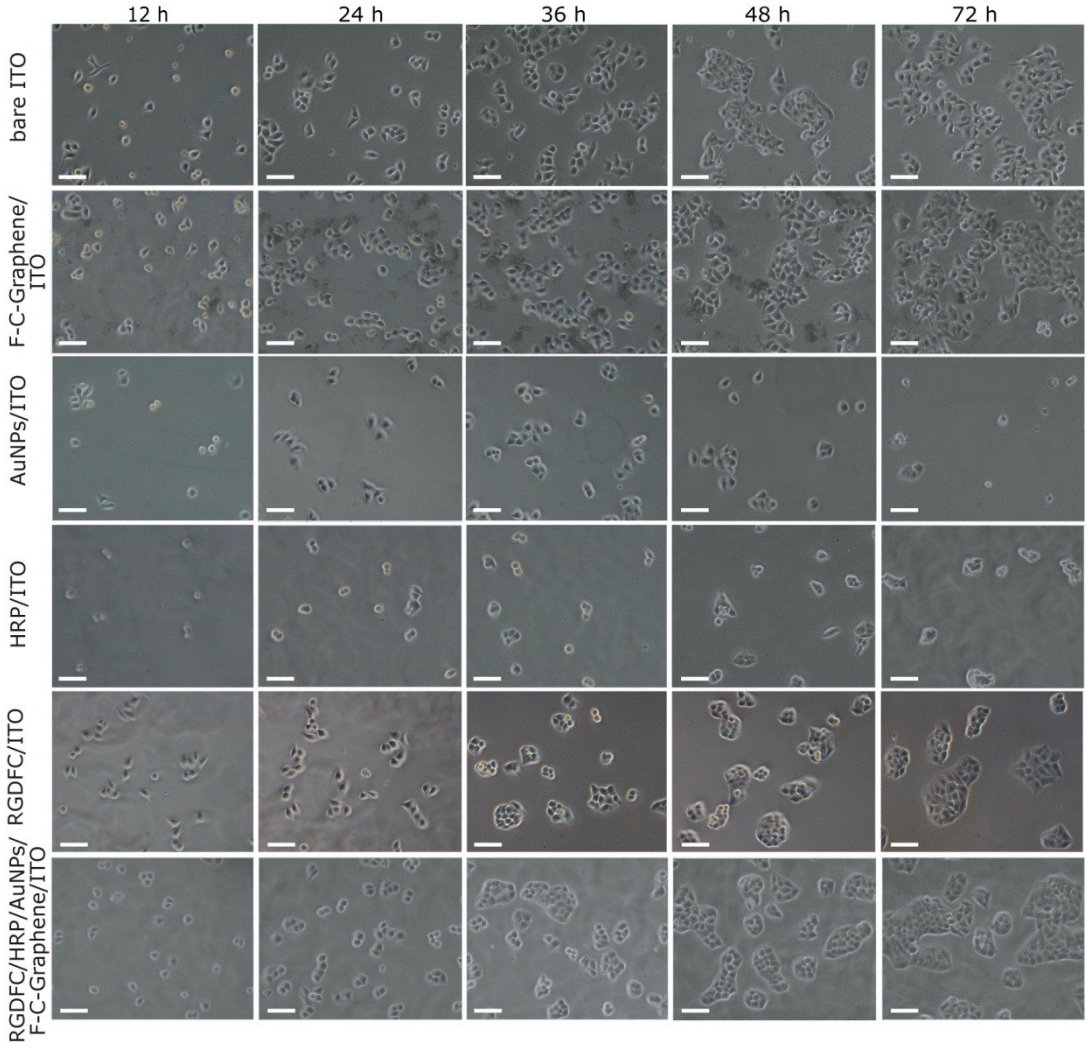

Fig. S4 Phase-contrast images of cells cultured on bare ITO, F-C-Graphene/ITO, AuNPs/ITO, HRP/ITO, RGDFC/ITO, and RGDFC/HRP/AuNPs/F-C-Graphene/ITO for 12, 24, 36, 48, and 72 h, respectively. Scar bars, 50 μm.

As shown in Fig. S4, the transparent property of the modified electrode allowed direct observation of the grown cells by an optical microscope. The flat two-dimensional bare ITO and F-C-Graphene/ITO surfaces displayed a good cell adhesion and proliferation behavior. But incorporation of AuNPs or HRP on ITO decreased the cell proliferation rate. After attaching RGDFC peptide, it was observed that Hela cells could adhere and proliferate normally from 0 to even 72 h on RGDFC/ITO and RGDFC/HRP/AuNPs/F-C-Graphene/ITO, which was attributed to the specific binding between integrin receptor on cells and RGDFC peptide. This result indicates

that the obtained RGDFC/HRP/AuNPs/F-C-Graphene/ITO interface can capture Hela cells and efficiently retain the viability of immobilized cells, showing its good biocompatibility.

6. Analysis of the morphological change of Hela cells after captured by the working electrode

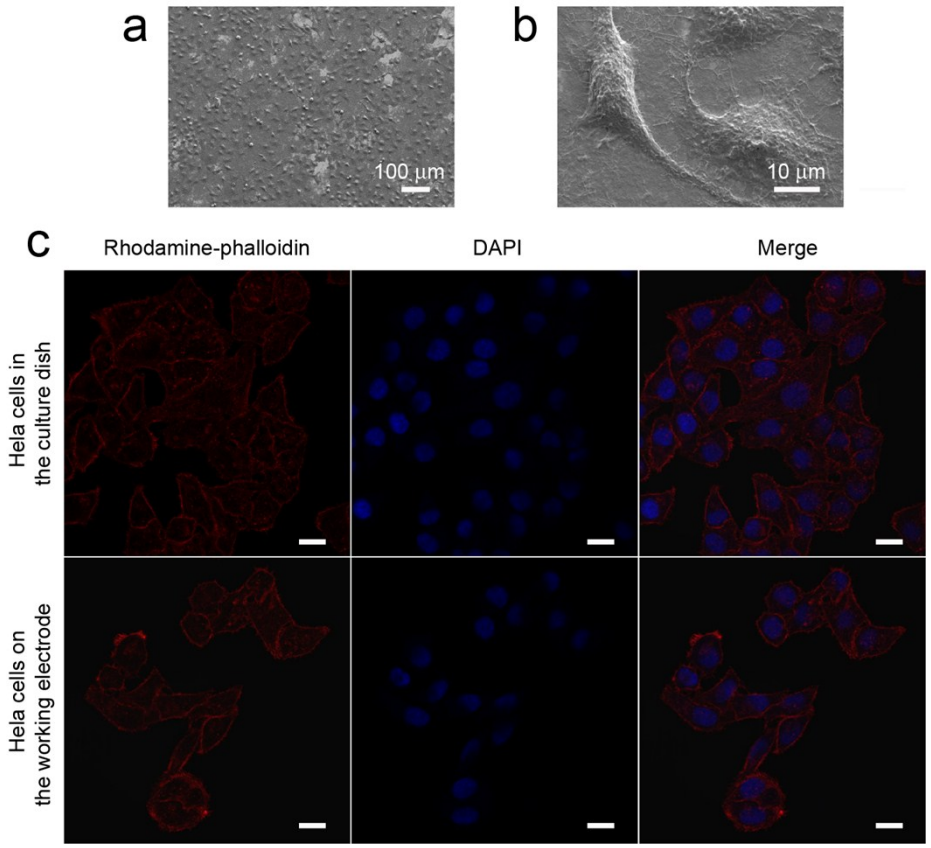

Fig. S5. Analysis of the morphological change of Hela cells after captured by the working electrode in the electrochemical cell using SEM and fluorescence staining. The large-scale (a) and zoomed-in (b) SEM images of Hela cells cultured on the working electrode, respectively. (c) The confocal microscope images of the HeLa cells in the culture dish (the first row) and on the working electrode (the second row), where the actin filaments and nucleus have been labeled with rhodamine phalloidin and DAPI, respectively. All scale bars, 20 μm.

7. References

[1] Y. Xu, H. Bai, G. Lu, C. Li, G. Shi, *J. Am. Chem. Soc. 2008*, **130**, 5856-5857.

[2] G. Frens, *Nature*, *1973*, **241**, 20-22.