analytical chemistry

Subscriber access provided by UNIV OF DURHAM

Article

A Novel Functional 3D Porous Conductive Polymer Hydrogels for Sensitive Electrochemiluminescence in situ Detection of H2O2 Released from Live Cells

Xinya Jiang, Huijun Wang, Ruo Yuan, and Yaqin Chai

Anal. Chem., **Just Accepted Manuscript** • DOI: 10.1021/acs.analchem.8b01168 • Publication Date (Web): 21 Jun 2018

Downloaded from http://pubs.acs.org on June 21, 2018

Just Accepted

"Just Accepted" manuscripts have been peer-reviewed and accepted for publication. They are posted online prior to technical editing, formatting for publication and author proofing. The American Chemical Society provides "Just Accepted" as a service to the research community to expedite the dissemination of scientific material as soon as possible after acceptance. "Just Accepted" manuscripts appear in full in PDF format accompanied by an HTML abstract. "Just Accepted" manuscripts have been fully peer reviewed, but should not be considered the official version of record. They are citable by the Digital Object Identifier (DOI®). "Just Accepted" is an optional service offered to authors. Therefore, the "Just Accepted" Web site may not include all articles that will be published in the journal. After a manuscript is technically edited and formatted, it will be removed from the "Just Accepted" Web site and published as an ASAP article. Note that technical editing may introduce minor changes to the manuscript text and/or graphics which could affect content, and all legal disclaimers and ethical guidelines that apply to the journal pertain. ACS cannot be held responsible for errors or consequences arising from the use of information contained in these "Just Accepted" manuscripts.

ACS Publications

is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

Published by American Chemical Society. Copyright © American Chemical Society. However, no copyright claim is made to original U.S. Government works, or works produced by employees of any Commonwealth realm Crown government in the course of their duties.

A Novel Functional 3D Porous Conductive Polymer Hydrogels for Sensitive Electrochemiluminescence in situ Detection of H_2O_2 Released from Live Cells

Xinya Jiang, Huijun Wang, Ruo Yuan*, Yaqin Chai*

Key Laboratory of Luminescent and Real-Time Analytical Chemistry (Southwest University), Ministry of Education, College of Chemistry and Chemical Engineering, Southwest University, Chongqing 400715, People's Republic of China

* Corresponding author. Tel.: +86-23-68252277; Fax: +86-23-68253172.

E-mail address: yuanruo@swu.edu.cn (R. Yuan), yqchai@swu.edu.cn (Y. Q. Chai)

Abstract

Reliable and sensitive in situ detection of molecule released from live cells attracts tremendous research interests as it shows significance in pathological and physiological investigation. In present work, a novel electrochemiluminescence (ECL) luminophore N-(aminobutyl)-N-(ethylisoluminol) functionalized Ag nanoparticles modified three dimensional (3D) polyaniline-phytic acid conducting hydrogels (ABEI-Ag@PAni-PA) are synthesized to adhere cells for in situ sensitive ECL detection of hydrogen peroxide (H_2O_2) released from live cells. The obtained 3D nanostructured ABEI-Ag@PAni-PA conducting hydrogels synergize the advantageous of conducting hydrogel and nanoparticle catalyst, in which the PAni-PA conducting hydrogels benefit the cell adhesion and high loading density of ABEI-Ag luminescent material due to its good biocompatibility, porous structure, and 3D continuous framework. Importantly, compared with traditional procedure for detection of H_2O_2 released from cells in solution, adhesion cells on ABEI-Ag@PAni-PA conducting hydrogels provides short diffusion distance to reaction sites for H_2O_2, thus realizing sensitive in situ monitoring of H_2O_2 released from cells under drug stimulation. With good biocompatibility, high sensitivity and easy preparation, the ECL biosensor based on ABEI-Ag@PAni-PA conducting hydrogels can be expanded to detect other molecules released from cells, which may facilitate the investigation of pathological and physiological.

Introduction

Hydrogen peroxide (H_2O_2), as one of the reactive oxygen species, is generated from oxygen metabolism in cells and closely associated with signal transduction and cell growth.[1,2] Mounting evidences indicate that H_2O_2 can be recognized as one of the biomolecule biomarkers in live cells for many diseases as overproduction of H_2O_2 could cause damage to nucleic, proteins, brain and tissues then lead to a series of diseases, such as Parkinson's disease and Alzheimer disease.[3-5] Therefore, it is essential to detect H_2O_2 quantitatively in cells for fully understanding its biological effects in cellular physiology. Currently, numerous sensors based on different analytical techniques, such as colorimetry,[6,7] electrochemical,[8,9] fluorescence,[10,11] chemiluminescence,[12] have been developed to detect H_2O_2 in cellular environment. However, cells were usually in solution due to the weak adhesion and poor biocompatibility of support materials for cells immobilization in most of these studies, resulting in a long diffusion distance to reaction sites for biomolecule which could further affect the detection sensitivity. Therefore, it is vital to explore some excellent biointerfaces with good cell adhesion, good biocompatibility, and good stability to detect biomolecular released from live cells sensitively.

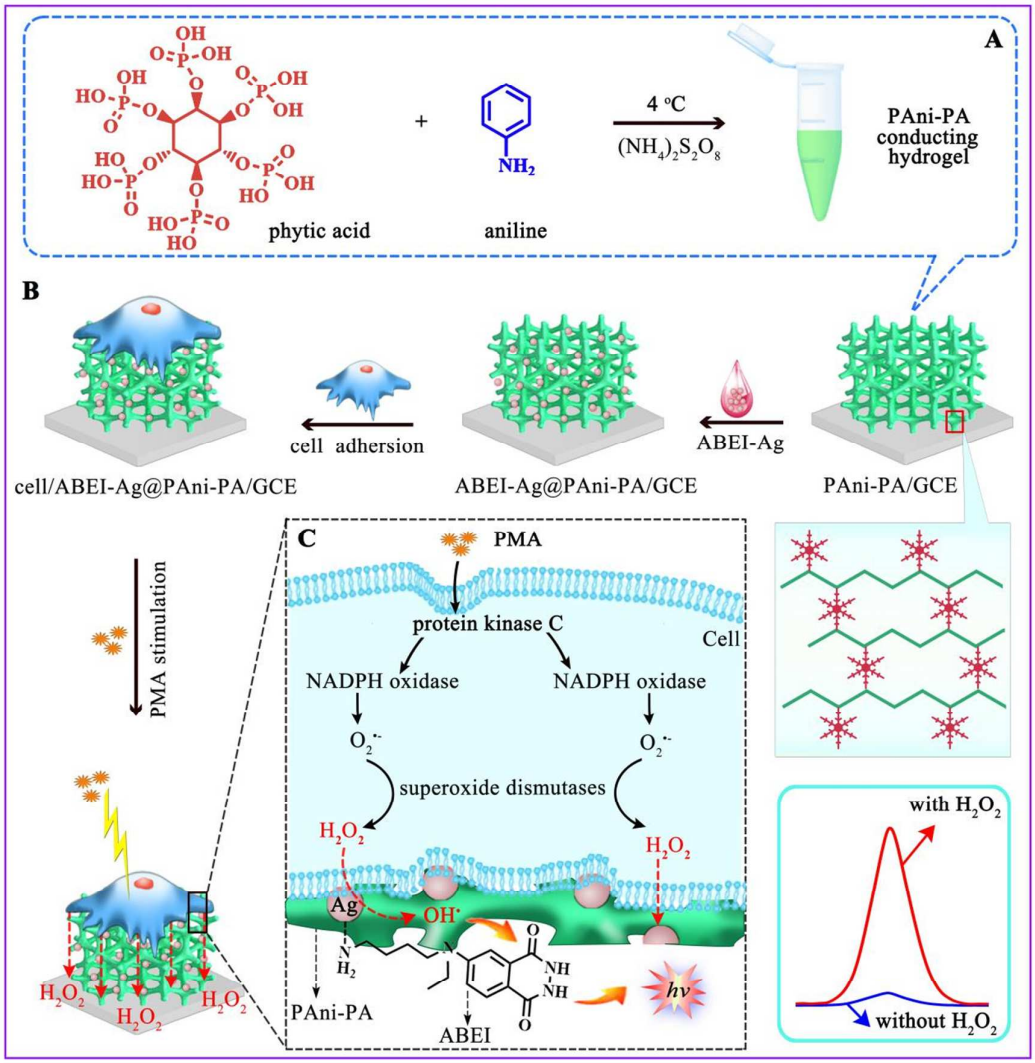
Due to the advantages of flexibility, good biocompatibility, mechanical stability and high permeability to biomolecules,[13] hydrogels have been applied in biosensing, drugs/enzymes release and so on.[14,15] Possessing excellent conductivity, good stability,

and easy of synthesis, conducting polymers are a class of polymer materials that are obtained by converting insulator polymer with conjugated π-bond to conductor by chemical/electrochemical doping methods.[16,17] Recently, conducting polymer hydrogels, which synergize the unique properties of hydrogels and organic conductors, are becoming as a unique material in the fields of supercapacitors and fuel cells.[18-20] It is worth mentioning that conducting polymer hydrogels possess the favorable features required by biosensor materials, such as good biocompatibility, high permeability to biomolecules and excellent conductivity.[21] Therefore, conducting polymer hydrogels can be applied as an ideal biosensor electrode substrate. It has been reported that the conductivity of polyaniline conducting hydrogels with phytic acid as gelator and dopant (PAni-PA) is higher than several other conducting polymer hydrogels such as polythiophene and polypyrrole.[22] In addition, the 3D structural hydrogels have been demonstrated to exhibit similarities to native tissues, rendering them as excellent extracellular matrix for cell adhesion.[23,24] Inspired by these advantages, PAni-PA hydrogels with high conductivity and good biocompatibility could be an extraordinary biointerface for in situ determination of H_2O_2 released from cells.

Electrochemiluminescence (ECL) technique holds the advantages of simplicity, stability, sensitivity, wide dynamic range, and near-zero background.[25,26] As one of the ECL reaction mechanisms, coreactant mechanism involves a bimolecular electrochemical reaction between the luminophor and a suitable coreactant.[27] Coincidentally, H_2O_2 has been demonstrated to be the most efficient and desirable

coreactant for luminophor luminol or its derivates.[28] Therefore, ECL technique can be used as a new, powerful and effective method for in situ determination of H_2O_2 released from live cells due to its unique advantages. Herein, we developed a novel ECL biosensor platform based on using ECL luminophore N-(aminobutyl)-N-(ethylisoluminol) functionalized Ag nanoparticles (AgNPs) decorated PAni-PA conducting hydrogels (ABEI-Ag@ PAni-PA) for cell adhesion and then achieved sensitive in situ detection of H_2O_2 released from live cells (Scheme 1). The PAni-PA conducting hydrogels provide the following outstanding advantages: (1) the 3D continuous framework of PAni-PA conducting hydrogels provide large space for the immobilization of ABEI-Ag ECL luminescent material to enhance ECL signal; (2) the PAni-PA conducting hydrogels exhibit facilitated charge transport and increased conductivity due to the formed long π-conjugated backbone, which was further conducive to the improvement of sensitivity. (3) the micron pores and good biocompatibility of PAni-PA conducting hydrogels endow them as excellent substrate for cell adhesion, providing a short diffusion distance. Additionally, AgNPs can catalyze H_2O_2 to decompose and generate hydroxyl radical ($OH^{\bullet}$),[29,30] which accelerate the ECL reaction of ABEI with high intensity. Upon the phorbol 12-myristate-13-acetate (PMA, H_2O_2 inducer[31]) stimulation, the proposed ECL biosensor realized sensitive in situ detection of H_2O_2 released from Hela cells as the released H_2O_2 could be immediately captured by ABEI-Ag due to the extremely short diffusion distance. This strategy using functional 3D porous conductive polymer

hydrogels to adhere cells directly provide a novel and sensitive method for in situ detection of biological molecules released from cells.



Scheme 1. The schematic illustration of (A) the fabrication procedure of the ABEI-Ag@PAni-PA conducting hydrogels, (B) the preparation of the ECL biosensor for cell assay, (C) the H_2O_2 production path triggered by PMA and ECL reaction mechanism.

Experimental Section

Reagents and Apparatus

N-(aminobutyl)-*N*-(ethylisoluminol) (ABEI) was purchased from J&K Technology Co., Ltd. (Beijing, China). Phytic acid (50%, w/w in water) was bought from Sigma (St. Louis, MO, U.S.A.). Aniline (99.5%) was obtained from Kelong Chemicals Ins. (Chengdu, China). Ethanol, $AgNO_3$, NaCl, KCl, glucose (Gl), ascorbic acid (AA), citric acid (CA), glutathione (GSH), nicotinamide adenine dinucleotide (NAD^+), nicotinamide adenine dinucleotide phosphate ($NADP^+$), $(NH_4)_2S_2O_8$ and H_2O_2 were obtained from Chemical Reagent Co. Ltd. (Chongqing, China). Phorbol 12-myristate-13-acetate (PMA) was purchased from Salomon biological reagent sales center (Tianjing, China). Fetal bovine serim (FBS) was purchased from Gibco Laboratories Life Technologies Inc. (Grand Island, NY). All the other chemicals were of analytical grade and used as received. Ultrapure water (18.2 MΩ cm resistivity) was used throughout the experiments. The phosphate buffer solution (PBS, 0.1 M, pH 8.0) was prepared by dissolving KCl, KH_2PO_4 and Na_2HPO_4 in ultrapure water, and NaOH or HCl was used to regulate the pH value.

A CHI 660D electrochemistry workstation (Shanghai Chenhua Instruments, China) was applied to measure electrochemical impedance spectroscopy (EIS). A multifunctional electrochemical and chemiluminescent analytical system of MPI-A model (Xi'an, China) was used to investigate electrochemiluminescence (ECL)

measurements. A conventional three-electrode system containing platinum wire as counter electrode, Ag/AgCl (sat. KCl) as reference electrode and modified glassy carbon electrode as working electrode was employed in all of the measurements. The microstructures of the as-prepared nanomaterials were measured by S-4800 scanning electron microscope (SEM, Hitachi, Japan). Elemental analysis of the as-prepared nanomaterials was investigated by X-ray photoelectron spectroscopy (XPS, Thermoelectricity Instruments, U.S.A.). In addition, the Fourier transform infrared spectroscopy (FTIR) was measured with Spectrum GX FTIR spectroscopy system (Perkin-Elmer, U.S.A.).

Preparation of ABEI-Ag ECL luminescent material

The ABEI-Ag ECL luminescent material was prepared according our previously published protocol.[32] Specifically, 1 mL of 10 mM $AgNO_3$ were added into a 7 mL of mixing solution ($V_{ethanol}$: $V_{ultrapure\ water}$ = 9 : 5). After stirring evenly, 0.5 mL of 20 mM ABEI solution were quickly added into the above solution, and then allowed it to react with stirring for overnight in darkness at room temperature. Finally, the ABEI-Ag ECL luminescent material could be obtained after centrifugation and washing for three times with use of ultrapure water.

Preparation of ABEI-Ag@PAni-PA conducting hydrogels modified electrode

The preparation of PAni-PA conducting hydrogels were according to the literature with a little modification.[33] Concretely, 0.460 mL of phytic acid and 0.115 mL of

aniline monomer were added into 1 mL of ultrapure water. After ultrasound, the mixture solution became to a clear and homogeneous solution and stored at 4 ^{o}C in a refrigerator. Simultaneously, 0.0715 g of $(NH_4)_2S_2O_8$ was added into 0.5 mL of ultrapure water. After fully dissolved, the $(NH_4)_2S_2O_8$ solution also stored at 4 ^{o}C in a refrigerator. After mixing the two solutions together, 4 μL of the mixed solution was immediately dropped on a cleaned glassy carbon electrode (GCE, Φ = 4 mm) which was polished intensively using alumina powder with 0.3 and 0.05 μm, respectively. Then, PAni-PA conducting hydrogels modified GCE could be obtained after reaction for 30 min at 4 ^{o}C. For purification of the PAni-PA conducting hydrogels modified GCE, the obtained GCE was immersed in ultrapure water for about 1 h to remove oligomers and excess ions. Subsequently, the prepared PAni-PA conducting hydrogels modified GCE was dried in a thermostat at 40 ^{o}C. Followed that, 20 μL of ABEI-Ag solution was dripped on PAni-PA conducting hydrogels modified GCE and reacted for overnight. Finally, the ABEI-Ag@PAni-PA conducting hydrogels modified GCE was prepared after washing with ultrapure water.

Cell culture and detection of H_2O_2 released from cells

Hela cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) under oxidative stress conditions supplemented with fetal bovine serum (10%) and then maintained in a humidified atmosphere of 5% CO_2 at 37 °C. Hela cells were collected and suspended into cell culture medium with 1×10^5 cells mL^{-1}. For ECL in situ

detection of H_2O_2 released from Hela cells, 20 μL of 1×10^5 cells mL^{-1} Hela cells was first dripped on ABEI-Ag@PAni-PA conducting hydrogels modified GCE. After cell adhesion for 2 h, the cells/ABEI-Ag@PAni-PA conducting hydrogels modified GCE was obtained and used as the working electrode for ECL detection of H_2O_2. After obtaining a steady ECL intensity from cells/ABEI-Ag@PAni-PA conducting hydrogels modified GCE, 20 μL of PMA (100 μg mL^{-1}) was added to 2 mL of PBS working buffer. Thus, H_2O_2 which is not only a product of oxygen metabolism could be effectively generated under PMA stimulation. The enhanced ECL signal indicated the amount of released H_2O_2 from Hela cells. Furthermore, a control experiment using ABEI-Ag@PAni-PA conducting hydrogels modified GCE was performed by adding same number of Hela cells and same concentration of PMA into 2 mL of PBS working buffer.

Results and discussion

Characterization of PAni-PA and ABEI-Ag@PAni-PA conducting hydrogels

Scanning electron microscopy (SEM) was utilized to characterize the morphologies of PAni-PA and ABEI-Ag@PAni-PA conducting hydrogels, as shown in Figure 1. Figure 1A and 1B revealed that the PAni-PA conducting hydrogels showed a porous 3D hierarchical microstructure, which was constructed with coral-like dendritic PAni-PA nanofibers with a uniform diameter of about 250 nm. Figure 1C and 1D showed the SEM images of ABEI-Ag@PAni-PA conducting hydrogels at different

dimensions. Figure 1C indicated that large number of ABEI-Ag nanoparticles were coated on the porous 3D PAni-PA conducting hydrogels homogeneously and densely. The magnified SEM image of Figure 1D revealed that the ABEI-Ag nanoparticles had a diameter of approximately 50 nm.

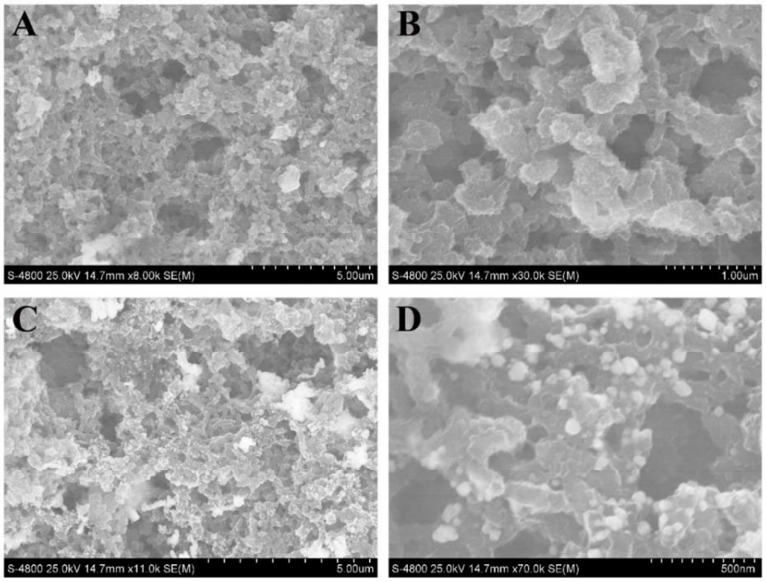


Figure 1. The SEM images of PAni-PA (A and B) and ABEI-Ag@PAni-PA conducting hydrogels (C and D) at different dimensions.

The chemical structure of PAni-PA and ABEI-Ag@PAn-PAi conducting hydrogels were analyzed by FTIR to prove their successful synthesis. The curve a and b in Figure 2 presented the FTIR spectra of PAni-PA and ABEI-Ag@PAni-PA conducting hydrogels, respectively. The peaks located at 1560 and 1478 cm^{-1} corresponded to the characteristic stretching vibrations of the quinoid ring and benzenoid ring. Besides, the peak at 1298 cm^{-1} was the absorption of aromatic amine Ar-N. The peaks at 797

and 1131 cm^{-1} were the benzene ring surface and in-plane bending vibration characteristic absorption band, respectively.[34] The peak at 505 cm^{-1} was caused by the aromatic ring bending vibration. The results indicated that the PAni-PA was successfully synthesized. Compared the FTIR spectra of ABEI-Ag@PAni-PA with that of PAni-PA, a new peak located at 3395 cm^{-1} was obtained, which corresponded to the absorption of primary amine group in ABEI. Therefore, the ABEI-Ag@PAni-PA conducting hydrogels were demonstrated to be successfully prepared.

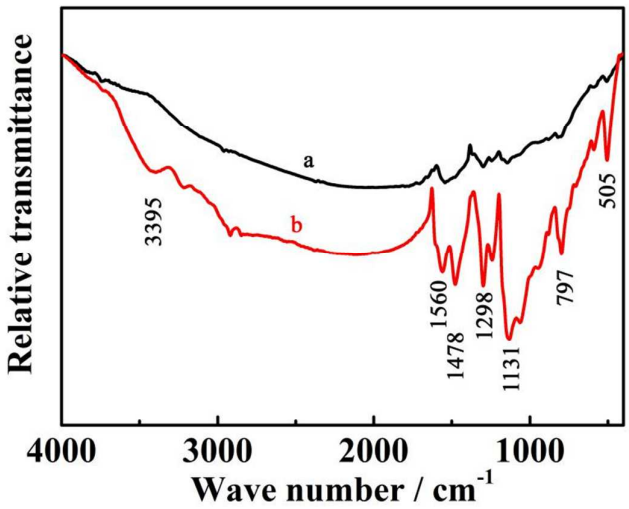


Figure 2. The FTIR spectra of PAni-PA (a) and ABEI-Ag@PAni-PA (b) conducting hydrogels.

In order to further prove the successful preparation of the ABEI-Ag@PAni-PA conducting hydrogels, XPS as a powerful technique was utilized to analyze the elemental component. Figure 3A presented the wide scan survey spectra of

ABEI-Ag@PAni-PA, in which the O 1s, N 1s, C 1s and Ag 3d were observed clearly at 532.11 eV, 399.41, 284.48 eV, and 368.26 eV, respectively. The N 1s, C 1s and Ag 3d XPS core level spectra of the obtained ABEI-Ag@PAni-PA were given in Figure 3B, 3C and 3D, respectively. The major peak line of N 1s could be decomposed into three peak lines (Figure 3B). The peaks located at 399.3 eV and 400.6 eV corresponded to the quinoid di-imine and benzenoid diamine nitrogen, and the peak centered at 402.3 eV was ascribed to positively charged nitrogen.[35] In addition, the major peak line of C 1s could be decomposed into four main peak lines (Figure 3C). The maximal peak at 284.6 eV was attributed to the C-C bond, the peak at 285.4 eV was assigned to C-N and C=N bonds, the peak at 286.5 eV was caused by C-O bond. The peak at 288.3 eV was the characteristic peak of C=O bond, which was attributed to the carbon atom in the carboxylic group (-COO-) of the ABEI oxidation product.[35,36] Furthermore, the decomposition peak lines of Ag 3d were showed in Figure 3D. The peaks centered at 367.4 eV and 373.4 eV corresponded to Ag^{+} $3d_{5/2}$ and Ag^{+} $3d_{3/2}$, while the peaks at 368.3 eV and 374.3 eV corresponded to Ag $3d_{5/2}$ and Ag $3d_{3/2}$, which was in agreement with previous reports.[37] Therefore, the results fully demonstrated that ABEI-Ag@PAni-PA conducting hydrogels were synthesized successfully.

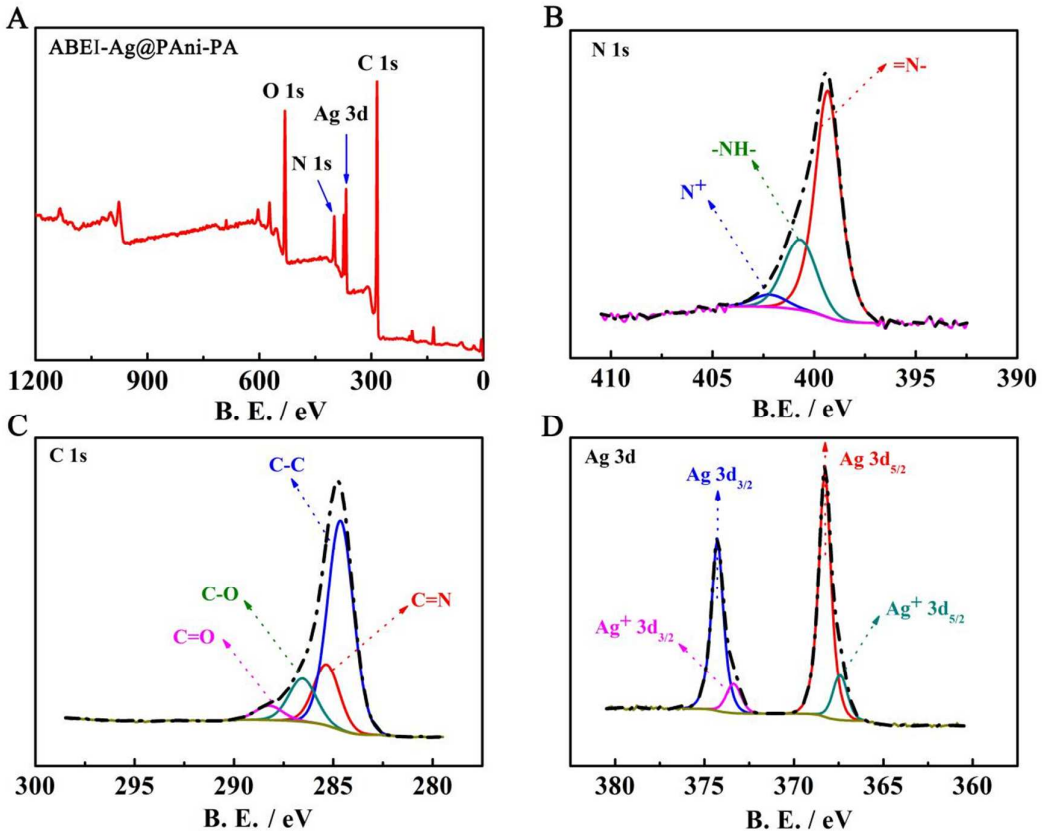


Figure 3. XPS wide scan spectrum of ABEI-Ag@PAni-PA conducting hydrogels (A), XPS narrow scans spectra of ABEI-Ag@PAni-PA for N 1s (B), C 1s (C), and Ag 3d (D).

Electrochemical characterization of ABEI-Ag@PAni-PA conducting hydrogels modified electrode

The conductivity of the PAni-PA and ABEI-Ag@PAni-PA conducting hydrogels were characterized by electrochemical impedance spectrum (EIS) in 5.0 mM $[Fe(CN)_6]^{4-/3-}$ containing 0.1 M KCl. Figure 4 was the Nyquist plot of the impedance curves obtained from bare GCE, PAni-PA, and ABEI-Ag@PAni-PA conducting

hydrogels modified GCE. Compared with the diameter of the semicircle obtained from bare GCE (curve a), the semicircle diameter of PAni-PA conducting hydrogels modified GCE decreased obviously (curve b), indicating that the PAni-PA conducting hydrogels had an excellent conductivity. However, the semicircle diameter of ABEI-Ag@PAni-PA conducting hydrogels modified GCE increased a little (curve c) compared with that of PAni-PA conducting hydrogels modified GCE. This phenomenon was caused by the negatively charged amino-phthalate ion analogue on the surface of AgNPs,[36] which may hinder the electron transfer in $[Fe(CN)_6]^{4-/3-}$ solution.

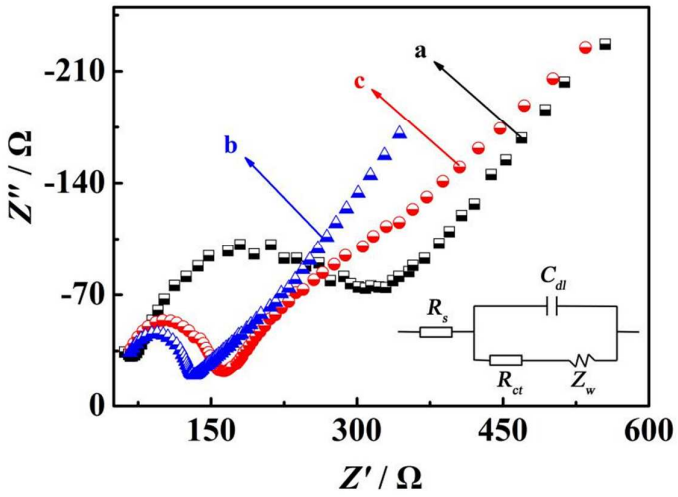


Figure 4. EIS of different materials modified GCE: (a) bare GCE, (b) PAni-PA modified GCE, and (c) ABEI-Ag@PAni-PA modified GCE. The frequency range was 1×10^{-2} – 1×10^{6} Hz.

Analytical performance of the ABEI-Ag@PAni-PA conducting hydrogels modified electrode for H_2O_2

To verify the sensitivity of ABEI-Ag@PAni-PA conducting hydrogels modified electrode toward H_2O_2, the ECL signals were recorded at different concentrations of H_2O_2 ranged from 0.01 to 40 μM. As showed in the Figure 5A, the ECL signal increased stepwise with the continuous addition of H_2O_2. Furthermore, two good linear segments were observed when plotting the ECL signal as a function of the H_2O_2 concentration (Figure 5B). The concentration for the first segment ranged from 0.01 to 1 μM, the linear equation was expressed as $I = 1240.6\ c + 129.0$ (I is the ECL intensity, c is the concentration of H_2O_2) with a regression coefficient (R) of 0.9918. The concentration for the second segment ranged from 1 to 40 μM, the linear equation was expressed as $I = 307.4\ c + 1068.5$ with a R of 0.9996. The detection limit was calculated to be 3.3 nM (S/N = 3). A comparison with other reported H_2O_2 sensors was displayed in Table 1, indicating that our proposed ECL biosensor had an excellent sensitivity for H_2O_2 determination.

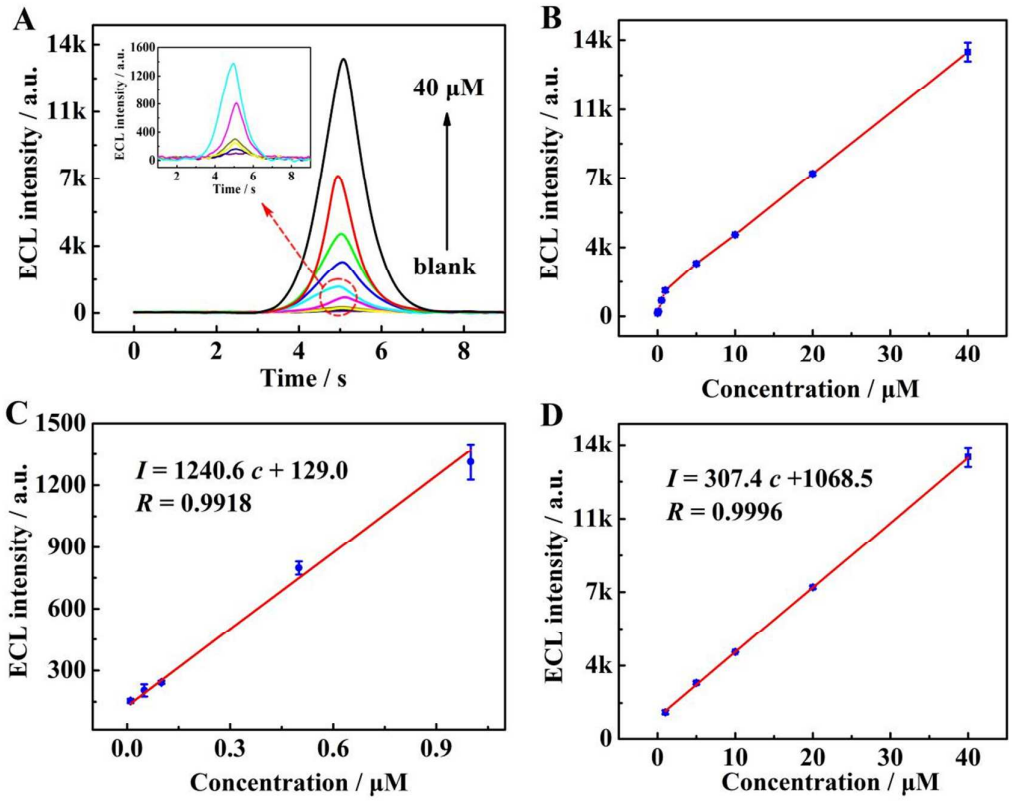


Figure 5. (A) The ECL response of the ABEI-Ag@PAni-PA modified GCE toward to the different concentrations of H_2O_2. (B) The calibration plot for H_2O_2 detection. (C) The calibration plot for H_2O_2 detection in the range of 0.01 to 1 μM. (D) The calibration plot for H_2O_2 detection in the range of 1 to 40 μM.

Table 1. Different methods for H_2O_2 detection.

method	Detection range	detection limit	refs.
fluorescence	0-10 μM	38 nM	38
colorimetry	50 nM-10 μM	22 nM	39
electrochemical	100 nM-2 μM	5 nM	40

electrochemical	1.0 μM -1.47 mM	370 nM	41
electrochemiluminescence	1-1000 μM	170 nM	42
electrochemiluminescence	0.01-40 μM	3.3 nM	this work

Other important parameters of ABEI-Ag@PAni-PA conducting hydrogels based ECL biosensor

The stability of the proposed ECL biosensor based on ABEI-Ag@PAni-PA conducting hydrogels was showed in Figure 6A. Upon the cyclic potential scan for 10 cycles, the ECL biosensor exhibited a comparatively stable signal with a relative standard deviation (RSD) of 0.97% in 10 μM of H_2O_2. This implied that the prepared biosensor had an excellent stability, which was presumably due to the intrinsic features of ABEI-Ag@PAni-PA conducting hydrogels. Furthermore, various interfering substances such as reactive molecules (GSH, NAD^+, $NADP^+$) and Na^+, K^+, Cl^-, AA, CA, Gl were utilized to investigate the antiinterference ability of the biosensor. As shown in Figure 6B, compared with 10 μM of H_2O_2, the concentration of Na^+, K^+, Cl^-, AA, CA, Gl, GSH, NAD^+, $NADP^+$ up to 1 mM did not cause any noticeable ECL signal, suggesting a good selectivity of the proposed ECL biosensor. In addition, reactive oxygen species (such as $O_2^{-\bullet}$, $OH^\bullet$) decomposed from H_2O_2, would interact with ABEI to produce excited-state ABEI which then emit light, thus the some reactive oxygen species in cells would impact the ECL signal. However, some literature indicated that the content of these reactive oxygen species in cells was little without drug stimulation[31,43,44], and the ECL signal obtained from the cells

without PMA stimulation in our study was almost the same with background signal (about 100 a.u.), indicating that the original molecules in cells did not affect the ECL signal. Therefore, the original molecules in cells did not affect the analysis result of H_2O_2 released from cells under PMA stimulation. As another important parameter of the biosensor, reproducibility was evaluated meticulously. Whether the same biosensor based on ABEI-Ag@PAni-PA conducting hydrogels was used to detect H_2O_2 for five times, or five biosensors based on ABEI-Ag@PAni-PA conducting hydrogels were utilized to detect H_2O_2, the obtained RSD were all lower than 5%. The results indicated that the proposed biosensor had an acceptable reproducibility for the detection of H_2O_2.

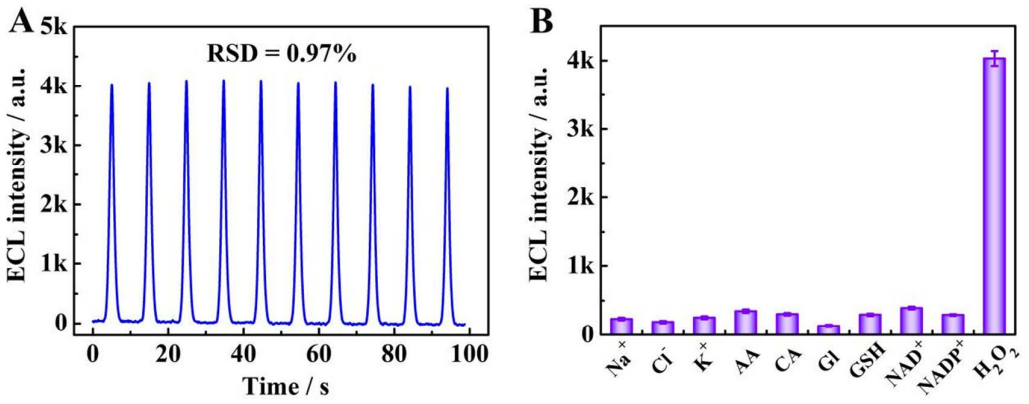


Figure 6. The stability (A) and selectivity (B) of ABEI-Ag@PAni-PA conducting hydrogels based ECL biosensor.

ECL in situ monitoring of H_2O_2 released from live cells

The practical application of the ECL biosensor based on ABEI-Ag@PAni-PA

conducting hydrogels was estimated by in situ quantitative detection of H_2O_2 released from Hela cells. The Hela cells were first adhered on the surface of ABEI-Ag@PAni-PA conducting hydrogels. Then, PMA as one of the H_2O_2 inducer was used to stimulate Hela cells. Subsequently, H_2O_2 was produced and directly captured by ABEI-Ag@PAni-PA conducting hydrogels sensing platform. As shown in Figure 7A, in the absence of PMA, the cell/ABEI-Ag@PAni-PA conducting hydrogels modified electrode showed a relatively low ECL signal (curve a). While an enhanced ECL signal was observed upon the injection and stimulation by PMA (curve b). The results indicated that the PMA-treated Hela cells produced H_2O_2, which then acted as coreactant of ABEI to enhance its ECL signal. The deductive mechanism for H_2O_2 production and ECL reaction mechanism were illustrated in Scheme 1C. According to the calibration equation obtained from ABEI-Ag@PAni-PA conducting hydrogels sensing platform and PMA-stimulated ECL signal response, the concentration of H_2O_2 released from Hela cells under the cell density of 1×10^5 cell mL^{-1} was calculated to be 0.16 μM. Therefore, known the cell density, total amount of released H_2O_2, and volume of electrolyte (2 mL), the average number of extracellular H_2O_2 molecules released from per cell (N_0) was calculated to be 0.96×10^{11} according to Avogadro equation ($n = N_0/N_A$, $N_A = 6.02\times10^{23}$ $mole^{-1}$),[44] which agreed with other reported methods.[45,46] Therefore, this analytical result may be taken as an accurate representation of H_2O_2 generation under oxidative stress conditions.

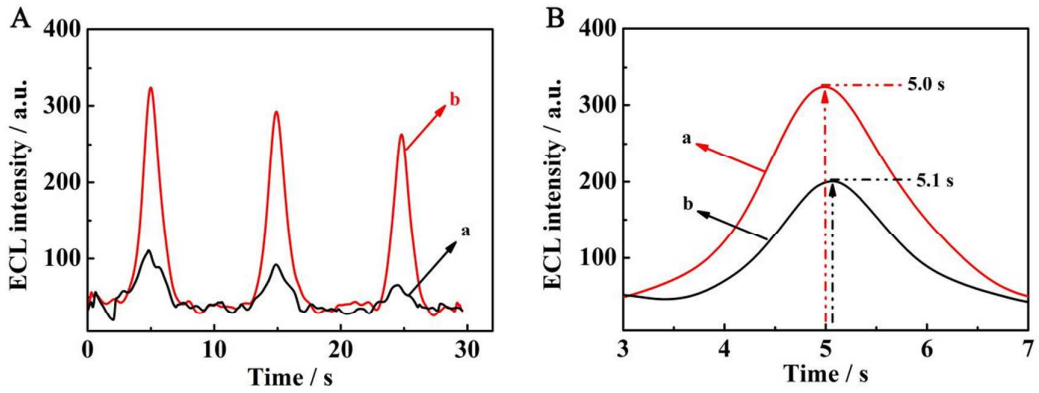


Figure 7. (A) The ECL responses of the cell/ABEI-Ag@PAni-PA/GCE without (a) and with (b) PMA stimulation. (B) ECL dynamic curve of (a) cells adhesion on ABEI-Ag@PAni-PA and (b) cells in solution.

In comparison, we used the ECL biosensor based on ABEI-Ag@PAni-PA conducting hydrogels to in situ detect H_2O_2 released from Hela cells by adding the same number of Hela cells and same concentration of PMA into the working buffer. As displayed in Figure 7B, the ECL intensity obtained from cells in solution (curve b) was lower than that obtained from cell adhesion on ABEI-Ag@PAni-PA conducting hydrogels (curve a). According to the obtained ECL intensity (Figure, 7B, curve b), the concentration of H_2O_2 released from Hela cells in solution was calculated as 0.07 μM, and the N_0 was calculated as 0.4×10^{11} which was lower than the proposed detection method. Furthermore, Figure 7B also indicated that when cells were adhered on ABEI-Ag@PAni-PA conducting hydrogels, the ECL peak appeared 0.1 s earlier than cells in solution. These results fully manifested that the H_2O_2 released from Hela cells in solution need longer time to diffuse through the medium to reach the sensing

interface, thus resulting in a low concentration on the sensing interface.

Conclusion

In summary, an ECL biosensor with high sensitivity was developed for in situ monitoring of H_2O_2 released from Hela cells using ABEI-Ag@PAni-PA conducting hydrogels as biointerface. The PAni-PA conducting hydrogels combined the advantages of conducting polymers and hydrogels, such as excellent conductivity, good biocompatibility, 3D porous structures, and good stability which endow them as excellent substrate for electrochemistry application, ABEI-Ag ECL luminescent material immobilization and cell adhesion. In addition, compared with traditional method for the detection of H_2O_2 released from cells in solution, adhesion cells on ABEI-Ag@PAni-PA conducting hydrogels could achieve more sensitive detection. Consequently, this strategy provided a direct avenue for cell adhesion and sensitive method for H_2O_2 detection, exhibiting great application potential in other intracellular biomolecules in situ detection and pathological investigation.

Author Information

* Corresponding authors:

Tel.: +86-23-68252277; Fax: +86-23-68253172.

E-mail address: yuanruo@swu.edu.cn (R. Yuan), yqchai@swu.edu.cn (Y. Q. Chai)

Acknowledgements

This work was financially supported by the NNSF of China (51473136, 21775124, 21675129, 21575116), the China-Singapore Plan (Chongqing) and the Chongqing Postdoctoral Science Foundation (Xm2017016), the Fundamental Research Funds for the Central Universities (XDJK2018AA003) and the China Postdoctoral Science Foundation (2016M602626), China.

References

(1) Miller, E. W., Dickinson, B. C., Chang, C. J. *Proc. Natl. Acad. Sci.* **2010**, 107, 15681-15686.

(2) Noh, J., Kwon, B., Han, E., Park, M., Yang, W., Cho, W., Yoo, W., Khang, G., Lee, D. *Nat. Commun.* **2015**, 6, 6907.

(3) Lin, M. T., Beal, M. F. *Nature* **2006**, 443, 787-795.

(4) Pramanik, D., Dey, S. G. *J. Am. Chem. Soc.* **2010**, 133, 81-87.

(5) Galaris, D., Skiada, V., Barbouti, A. *Cancer Lett.* **2008**, 266, 21-29.

(6) Chen, S., Hai, X., Chen, X. W., Wang, J. H. *Anal. Chem.* **2014**, 86, 6689-6694.

(7) Farrokhnia, M., Karimi, S., Momeni, S., Khalililaghab, S. *Sens. Actuators B Chem.* **2017**, 246, 979-987.

(8) Sun, Y. J., Luo, M. C., Meng, X. X., Xiang, J., Wang, L., Ren, Q. S., Guo, S. J. *Anal. Chem.* **2017**, 89, 3761-3767.

(9) Li, Z. Z., Xin, Y. M., Wu, W. L., Fu, B. H., Zhang, Z. H. *Anal. Chem.* **2016**, 88, 7724-7729.

(10) Ren, M. G., Deng, B. B., Wang, J. Y., Kong, X. Q., Liu, Z. R., Zhou, K., He, L. W., Lin, W. Y. *Biosens. Bioelectron*. **2016**, 79, 237-243.

(11) Ren, M. G., Deng, B. B., Zhou, K., Kong, X. Q., Wang, J. Y., Lin, W. Y. *Anal. Chem.* **2017**,

89, 552-555.

(12) Zhao, C. X., Cui, H. B., Duan, J., Zhang, S. H., Lv, J. G. *Anal. Chem.* **2018**, 90, 2201-2209.

(13) Kopeček, J. *Nature* **2002**, 417, 388-391.

(14) Cai, Z. Y., Kwak, D. H., Punihaole, D., Hong, Z. M., Velankar, S. S., Liu, X. Y., Asher, S. A. *Angew. Chem. Int. Ed.* **2015,** 54, 13036-13040.

(15) Gupta, M. K., Martin, J. R., Werfel, T. A., Shen, T. W., Page, J. M., Duvall, C. L. *J. Am. Chem. Soc.* **2014**, 136, 14896-14902.

(16) Janata, J., Josowicz, M. *Nat. Mater.* **2003**, 2, 19-24.

(17) Bryan, A. M., Santino, L. M., Lu, Y., Acharya, S., D'Arcy, J. M. *Chem. Mater.* **2016**, 28, 5989-5998.

(18) Hao, G. P., Hippauf, F., Oschatz, M., Wisser, F. M., Leifert, A., Nickel, W., Mohamed-Noriega, N., Zheng, Z. K., Kaskel, S. *ACS Nano* **2014**, 8, 7138-7146.

(19) Shi, Y., Zhou, X. Y., Zhang, J., Bruck, A. M., Bond, A. C., Marschilok, A. C., Takeuchi, K. J., Takeuchi, E. S., Yu, G. H. *Nano Lett.* **2017**, 17, 1906-1914.

(20) Zhao, F., Shi, Y., Pan, L. J., Yu, G. H. *Acc. Chem. Res.* **2017**, 50, 1734-1743.

(21) Åsberg, P., Inganäs, O. *Biosens. Bioelectron.* **2003**, 19, 199-207.

(22) Pan, L. J., Yu, G. H., Zhai, D. Y., Lee, H. R., Zhao, W. T., Liu, N., Wang, H. L., Tee, B. C.-K., Shi, Y., Cui, Y., Bao, Z. N. *Proc. Natl. Acad. Sci.* **2012**, 109, 9287-9292.

(23) Wu, Y. B., Wang, L., Guo, B. L., Ma, P. X. *ACS Nano* **2017**, 11, 5646-5659.

(24) Lou, J. Z., Stowers, R., Nam, S. M., Xia, Y., Chaudhuri, O. *Biomaterials* **2018**, 154, 213-222.

(25) Richter, M. *Chem. Rev.* **2004**, 104, 3003-3036.

(26) Li, L. L., Chen, Y., Zhu, J. J. *Anal. Chem.* **2016**, 89, 358-371.

(27) Valenti, G., Rampazzo, E., Bonacchi, S., Petrizza, L., Marcaccio, M., Montalti, M., Prodi, L., Paolucci, F. *J. Am. Chem. Soc.* **2016**, 138, 15935-15942.

(28) Kitte, S. A., Gao, W. Y., Zholudov, Y. T., Qi, L. M., Nsabimana, A., Liu, Z. Y., Xu, G. B. *Anal. Chem.* **2017**, 89, 9864-9869.

(29) Wang, C. I., Chen, W. T., Chang, H. T. *Anal. Chem.* **2012**, 84, 9706-9712.

(30) He, Y., He, X., Liu, X. Y., Gao, L. F., Cui, H. *Anal. Chem.* **2014**, 86, 12166-12171.

(31) Dong, B. L., Song, X. Z., Kong, X. Q., Wang, C., Tang, Y. H., Liu, Y., Lin, W. Y. *Adv. Mater.* **2016**, 28, 8755-8759.

(32) Jiang, X. Y., Wang, H. J., Wang, H. J., Yuan, R., Chai, Y. Q. *Anal. Chem.* **2016**, 88, 9243-9250.

(33) Li, L. L., Wang, Y. Q., Pan, L. J., Shi, Y., Cheng, W., Shi, Y., Yu, G. H. *Nano Lett.* **2015**, *15*, 1146-1151.

(34) Mai, L. Q., Xu, X., Han, C. H., Luo, Y. Z., Xu, L., Wu, Y. A., Zhao, Y. L. *Nano Lett.* **2011**, 11, 4992-4996.

(35) Wu, G., Li, L., Li, J. H., Xu, B. Q. *J. Power Sources* **2006**, 155, 118-127.

(36) He, Y., Liu, D. Q., He, X. Y., Cui, H. *Chem. Commun.* **2011**, 47, 10692-10694.

(37) Jiang, P., Li, S. Y., Xie, S. S., Gao, Y., Song, L. *Chem. Eur. J.* **2004**, 10, 4817-4821.

(38) Gao, C. C., Tian, Y., Zhang, R. B., Jing, J., Zhang, X. L. *Anal. Chem.* **2017**, 89, 12945-12950.

(39) Mao, X. X., Chen, G. F., Wang, Z. H., Zhang, Y. G., Zhu, X. L., Li G. X. *Chem. Sci.* **2018**, 9, 811-818.

(40) Sun, X. L., Guo, S. J., Liu, Y., Sun, S. H. *Nano. Lett.* **2012**, 12, 4859-4863.

(41) Cheng, C. F., Zhang, C. M., Gao, X. H., Zhuang, Z. H., Du, C., Chen, W. *Anal. Chem.* **2018**, 90, 1983-1991.

(42) Qi, L. M., Xia, Y., Qi, W. J., Gao, W. Y., Wu, F. X., Xu, G. B. *Anal. Chem.* **2016**, 88, 1123-1127.

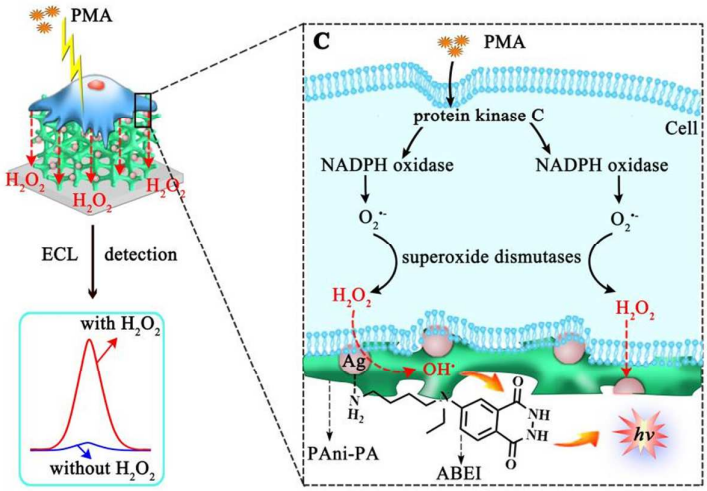
(43) Zhuang, M., Ding, C. Q., Zhu, A. W., Tian, Y. *Anal. Chem.* 2014, 86, 1829-1836.

(44) Hu, F. X., Kang, Y. J., Du, F., Zhu, L., Xue, Y. H., Chen, T., Li, C. M. *Adv. Funct. Mater.* **2015**, 25, 5924-5932.

(45) Lian, M. L., Chen, X., Lu, Y. L., Yang, W. S. *ACS Appl. Mater. Interfaces* **2016**, 8, 25036-25042.

(46) Guo, C. X., Zheng, X. T., Lu, Z. S., Lou, X. W., Li, C. M. *Adv. Mater.* **2010**, 22, 5164-5167

For TOC Only


PMA
H_2O_2
H_2O_2
H_2O_2
ECL
detection
with H_2O_2
without H_2O_2
C
PMA
protein kinase C
Cell
NADPH oxidase
NADPH oxidase
$O_2^{\cdot-}$
$O_2^{\cdot-}$
superoxide dismutases
H_2O_2
H_2O_2
Ag
OH·
NH_2
NH
NH
O
O
hv
PAni-PA
ABEI