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Self-assembled peptide hydrogel as a smart biointerface for enzyme-based electrochemical biosensing and cell monitoring

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ABSTRACT

A self-assembled peptide nanofibrous hydrogel composed of N-fluorenylmethoxycarbonyl-diphenylalanine (Fmoc-FF) was used to construct a smart biointerface. This biointerface was then used for enzyme-based electrochemical biosensing and cell monitoring. The Fmoc-FF hydrogel had two functions. One was as a matrix to embed an enzyme model, horseradish peroxidase (HRP), during the self-assembly of Fmoc-FF peptides. The other was used as a robust substrate for cell adhesion. Experimental data demonstrated that HRP was immobilized in a stable manner within the peptide hydrogel, and that HRP retained its inherent bioactivity toward H_2O_2. The HRP also can realize direct electron transfer in the Fmoc-FF hydrogel. The resulting third-generation electrochemical H_2O_2 biosensor exhibited good analytical performance, including a low limit of detection of 18 nM, satisfactory reproducibility, and high stability and selectivity. HeLa cells were then adhered to the HRP/Fmoc-FF hydrogel-modified electrode. The sensitive in situ monitoring of H_2O_2 released from HeLa cells was realized. This biointerface based on the Fmoc-FF hydrogel was easily prepared; environmentally friendly, and also versatile for integration of other cells adhesion and recognized molecules to monitoring various cellular biomolecules. The smart biointerface has potential application in broad physiological and pathological investigations.

KEYWORDS

Peptide hydrogel; Self-assembly; Electrochemical biosensing; Cell monitoring; H_2O_2

INTRODUCTION

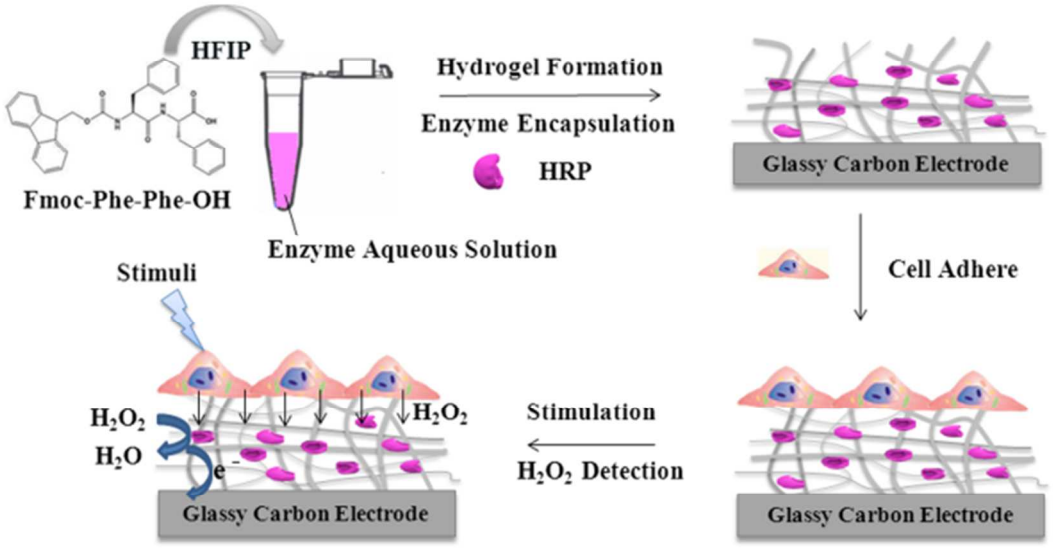
In situ molecular detection based on the cell experiment has basic meaning as well as practical significance in cell function, physiology, pathology and toxicology.[1–3] The design and fabrication of functional biointerfaces are vital in this field. The adhesion of cells and the detection performance of the biointerface should also be considered.[4] Various materials including inorganic nanomaterials and organic polymers have been used to build functional biointerfaces. However, there are still some drawbacks. For example, procedures for fabricating some interfaces are cumbersome and time-consuming.[5,6] The biocompatibility of some nanomaterials remains uncertain, and the mechanical stability of conventional hydrogels is poor.[7,8] The weak adhesion and incompatible dimensions of many supports for immobilizing living cells do not meet application requirements.[9] The sensitivity and selectivity for target analytes also requires improvement, because of the low amount of analyte usually released from cells.[10] Smart biointerfaces for the in situ quantitative and selective detection of cells are therefore required. They should exhibit good cell adhesion, dimensional compatibility, high sensitivity, and be easily prepared, biocompatible, and stable.

Self-assembly based on the simple peptide, diphenylalanine (FF), has received much recent attention.[11] This peptide and its derivate can self-organize into various structures under mild conditions, via an synergy of hydrogen-bond interaction and π–π stacking between benzene rings.[12] These structures exhibit interesting optical, mechanical, and electrochemical properties.[13–15] Especially, Fmoc-FF can easily form a transparent and hard hydrogel.[16] Compared with other synthetic polymers, the Fmoc-FF hydrogel exhibits

the following attractive features: (1) its preparation via self-assembly does not need strict factor for instance high pressures and temperatures;[17] (2) its structure is a three-dimensional (3D) network which is composed of nanofibers and has a large amount of micropores;[18] (3) its mechanical stability is good, and is considerably stronger and stiffer than other peptide or protein hydrogels;[19] (4) its biocompatibility and adhesion are excellent, and are capable of supporting cell culture;[20] (5) its supramolecular π-stacking backbone formed from electron-conducting aromatic residues may provide effective intermolecular electron delocalization, which would facilitate charge transport and increase conductivity.[21] Thus, this hydrogel has potential as a cost-effective scaffold for 3D cell culture, the fabrication of advanced biosensors, and controlled drug release. As far as we know, few papers have investigated enzyme immobilization and cell adhesion by the Fmoc-FF hydrogel, for the in situ detection of cellular biomolecules.

Herein, the Fmoc-FF hydrogel is used as a biointerface, and an enzyme-based electrochemical biosensor and cell monitoring are demonstrated for the first time (Scheme 1). In the process of the self-assembly of Fmoc-FF, the model enzyme horseradish peroxidase (HRP) was encapsulated within the hydrogel matrix. This allowed the detection of H_2O_2 released from living cells. Spectroscopic measurements suggested that HRP immobilized within the Fmoc-FF hydrogel retained its biological activity. HRP achieved the direct electron transfer within the hydrogel. These findings constitute the fabrication of a third-generation electrochemical biosensor for detecting H_2O_2, which is based on the Fmoc-FF hydrogel. The good analytical performance for sensing H_2O_2 was evidenced by its high selectivity and low detection limit. Living cells were then adhered

to the surface of a Fmoc-FF hydrogel-modified electrode, and the in situ monitoring of H_2O_2 released from HeLa cells was realized. It is important that the current Fmoc-FF hydrogel-based biosensor is low-cost, easily prepared, and environmentally friendly. It potentially allows for the integration of other cells and enzymes for realizing the real-time monitoring of various cellular biomolecules.



Scheme 1. Schematic diagram of the construction of the biointerface towards enzyme-based electrochemical biosensing and cell monitoring.

EXPERIMENTAL

Materials

N-Fluorenylmethoxycarbonyl diphenylalanine (Fmoc-FF) was purchased from Bachem (Bubendorf, Switzerland). 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) and

2,2-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) were obtained from Sigma-Aldrich. Horseradish peroxidase (HRP, RZ≥3, activity≥250 units mg^{-1}) was from Shanghai Xueman Biotechnology Co. Ltd. (China). Hydrogen peroxide (H_2O_2, 30%) was supplied with Beijing Chemical Works (China). The solutions were 0.1 M of phosphate buffer solution (PBS, pH 7.0) in the electrochemical test.

Preparation of HRP/Fmoc-FF hydrogel

A Fmoc-FF stock solution was freshly provided through dissolving the freeze-dried powder of Fmoc-FF in HFIP (100 mg mL^{-1}) and then ultrasonicated until obtained a clear solution. Subsequently, the stock solution was diluted with pure doubly distilled water (DDW) containing 1 mg mL^{-1} HRP to a final concentration (10 mg mL^{-1}). After a few minutes, the Fmoc-FF hydrogels were formed. The hydrogel with encapsulating enzyme molecules was denoted as HRP/Fmoc-FF hydrogel.

Preparation of the modified electrodes

Firstly, using alumina slurry polished glassy carbon electrodes (GCE, diameter 3 mm). Afterwards the GCE washed with DDW, sonicated and then dried. An aliquot (6 μL) of HRP/Fmoc-FF hydrogel was then dropped on the clean GCE and dried at 4 °C for 12 h. The modified electrode denoted as HRP/Fmoc-FF hydrogel/GCE. A Fmoc-FF hydrogel/GCE was constructed using the same steps.

Cell culture and in situ detection of H_2O_2 released from living cells

HeLa cells were grown within replicate 96-well microliter plates under a humidified atmosphere in Dulbecco's Modified Eagle's Medium (DMEM). Then, the cells were collected by centrifugation and suspended into the cell culture medium. 6 μL cell

suspensions (5×10^6 cells mL^{-1}) were dropped on the HRP/Fmoc-FF hydrogel/GCE. Afterwards this electrode was maintained in an incubator for 2 h in order to the adhesion of the cells on the HRP/Fmoc-FF hydrogel/GCE. The amperometric detection of H_2O_2 released from living cells was performed using the cells/HRP/Fmoc-FF hydrogel/GCE as the working electrode. After getting the stable electrode current, 10 μL 100 μg mL^{-1} PMA was injected to PBS (1 mL). The increase of the current was monitored to indicate the amount of H_2O_2 released from cells adhered on the HRP/Fmoc-FF hydrogel/GCE. In addition, a control experiment at the HRP/Fmoc-FF hydrogel/GCE was investigated by adding the HeLa cells (2×10^6 cells mL^{-1}) into the measured PBS. The response change for H_2O_2 released from cells was also recorded by adding the same amount PMA into PBS (1 mL).

Leakage tests for HRP from hydrogel and measurement of the enzymatic activity

In order to explore the leakage of HRP from the hydrogel, 1.5 mL DDW was added to the equal volume of peptide hydrogel encapsulated HRP. Then the supernatant was collected and recorded the PL intensity. From this, the amount of HRP leakage was estimated. The enzymatic activities for Fmoc-FF hydrogel immobilized HRP and free HRP were measured the PL intensity with 4 mM ABTS, 3.3 mM H_2O_2, 0.010 μM enzyme in 3 ml water.

MTT assay

Cells were cultured for 3 days. Determined by MTT reagent into the cells and incubated with 4 h. Then through dissolving acidified isopropanol to remove the culture medium and the reduced MTT reagent. The viability of cells was evaluated via testing the

absorbance employing a microplate system.

Instruments and measurements

Scanning electron microscopy (SEM) images were acquired with a Zeiss Supra 55 scanning electron microscope, and the accelerating voltage is 20 kV. A Hitachi H-800 instrument (Japan) was used for examining Transmission electronic microscopy (TEM) images. The UV–vis absorption spectra were obtained from a UV–vis spectrophotometer (Perkin-Elmer Lambda 35). A CHI 660B electrochemical workstation (Shanghai CH Instruments, China) was used to perform electrochemical measurements. The working electrode, counter electrode and reference electrode is modified GCE, platinum wire and Ag/AgCl electrode, respectively. The working solutions were removed oxygen by purifying with nitrogen before testing for 20 minutes, and maintained the nitrogen environment in the process of the electrochemical measurements. The cyclic voltammograms (CVs) test is applied at a scan rate of 0.1 V s^{-1}, an applied potential of –0.35 V was used to carry out the current-time measurement.

RESULTS AND DISCUSSION

Characterization of HRP/Fmoc-FF hydrogel

Figure 1A and 1D show optical pictures of the peptide hydrogel and HRP/peptide hydrogel contained in inverted vials, respectively. No gravitational flow was observed within the inverted vials, confirming that gels could be formed regardless of the presence of HRP. It can be seen that the Fmoc-FF hydrogel was colorless and translucent, the HRP/Fmoc-FF hydrogel had a uniform light-brown appearance due to HRP having a

red-brown color. This indicated that HRP was incorporated and well distributed within the hydrogel. The SEM images in Figure 1B and 1E indicated that the Fmoc-FF hydrogels with and without HRP had similar porous 3D networks, which consisted of numerous nanofibers of diameters of approximately 30–40 nm. These fiber diameters were just within the scope observed for extracellular matrix (5–300 nm), suggesting that the HRP/Fmoc-FF hydrogel shows potential to be a scaffold material for cell cultures.[16] The TEM images in Figure 1C and 1F indicated that the two hydrogels were composed of interlaced nanofibers with some pores. The degree of cross-linking of the peptide nanofibers was more pronounced in the HRP/peptide hydrogel (Figure 1F) than in the Fmoc-FF hydrogel (Figure 1C). This may have resulted from enzyme surface functional groups such as $–NH_2$ interacting with the peptide nanofibers, thus leading to better adhesion between nanofibers. The interaction promoted the entrapment of HRP molecules within the Fmoc-FF hydrogel matrix. The HRP/Fmoc-FF hydrogel retained its porous nanoscale framework, enabling a high surface area and potentially the efficient mass transport of analytes and products. This could potentially accelerate the response time and increase the sensitivity of biosensors upon the hydrogel.

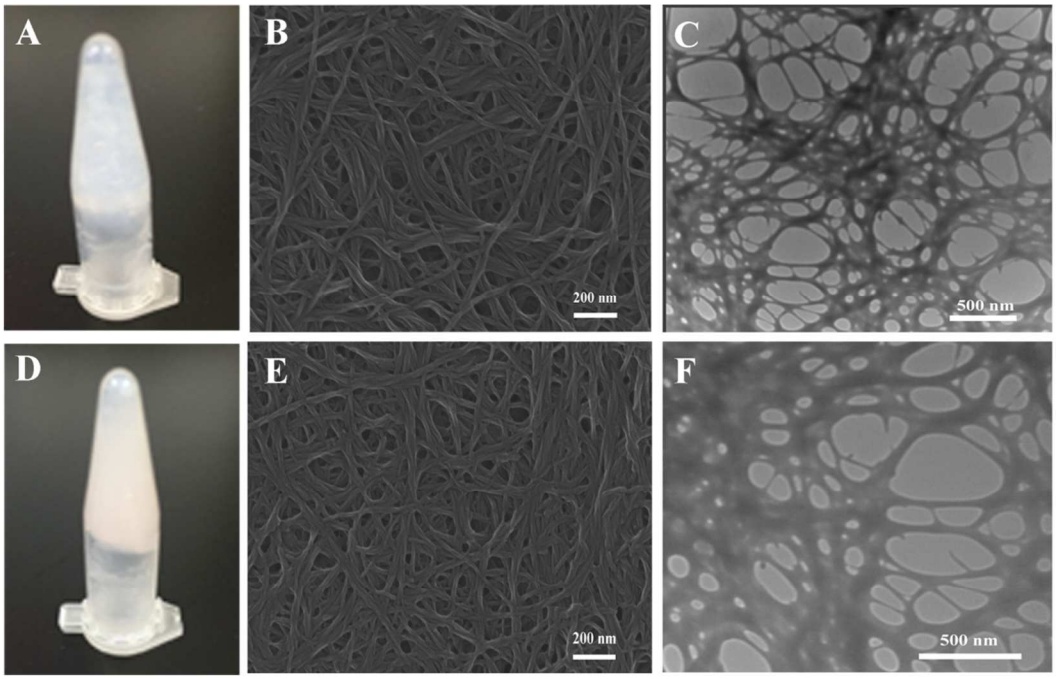


Figure 1. (A) Optical, (B) SEM, and (C) TEM images of the Fmoc-FF hydrogel; (D) Optical, (E) SEM, and (F) TEM images of the HRP/Fmoc-FF hydrogel.

Ultraviolet-visible (UV-vis) absorption spectroscopy was then applied to investigate the construction of HRP encapsulated within the Fmoc-FF hydrogel. The location of the Soret absorption peak of heme offers message about the conformational integrity of the protein.[22] The UV-vis spectra of peptide hydrogel, HRP, as well as HRP/Fmoc-FF hydrogel were shown in Figure 2. No absorption was observed in Fmoc-FF peptide hydrogel (Figure 2a). HRP entrapped in the Fmoc-FF hydrogel gave rise to a characteristic absorption peak at 403 nm (Figure 2c),[23] which was comparable to that observed for solution-state HRP (Figure 2b). This meant that HRP kept the substantive features of its original formation after being immobilized within the Fmoc-FF hydrogel.

In addition, through leakage tests for HRP from hydrogel and measurement of the enzymatic activity, we known that more than 96% HRP was steadily encapsulated in the network of the peptide hydrogel, and the enzymatic activity retained after immobilization, indicating that the peptide hydrogel can be availably applied to trapping enzyme and keeping the enzymatic activity.

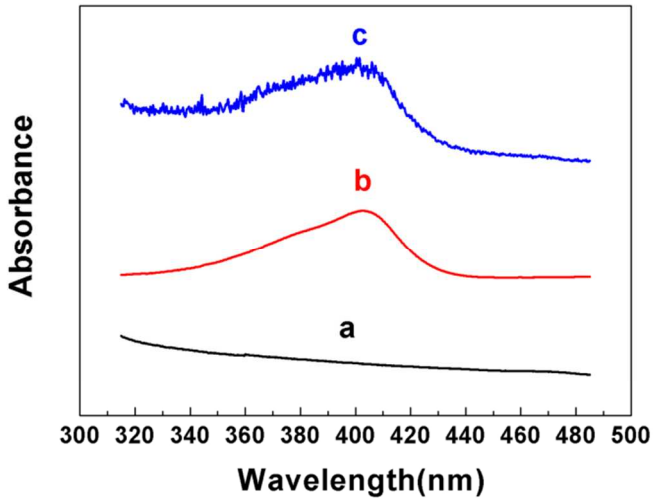


Figure 2. UV-vis spectra of (a) Fmoc-FF hydrogel, (b) HRP, and (c) the HRP/Fmoc-FF hydrogel.

Direct electrochemistry of HRP/Fmoc-FF hydrogel/glassy carbon electrode

Figure 3A shows typical CVs of various electrodes in 0.1 M PBS. No redox peaks were seen for the Fmoc-FF hydrogel/GCE (curve a). The HRP/Fmoc-FF hydrogel/GCE (curve b) exhibited a couple of redox peaks at –0.358 and –0.314 V, which were feature of the Fe(III)/Fe(II) redox couple of the HRP heme prosthetic group. This indicating that

HRP realized the direct electron transfer. The formal potential of the HRP/Fmoc-FF hydrogel/GCE was –0.336 V, which is similar to values observed at other modified electrode surfaces.[24,25]

Figure 3B showed the effect of different scan rates on the electrochemical response of the HRP/Fmoc-FF hydrogel/GCE. It can be seen that the anodic and cathodic peak currents increased linearly with scan rates, and the redox process was a typical quasi-reversible process. The regression equation was derived as I_{pc} (μA) = –1.827 v (V s^{-1}) +0.334. According to the report in the previous literature,[26,27] the derived number of electrons n is 0.94, and the electron transfer rate constant (Ks) is 1.07 s^{-1}. It turned out that the electron transfer is a surface–controlled electrochemical process.[28,29]

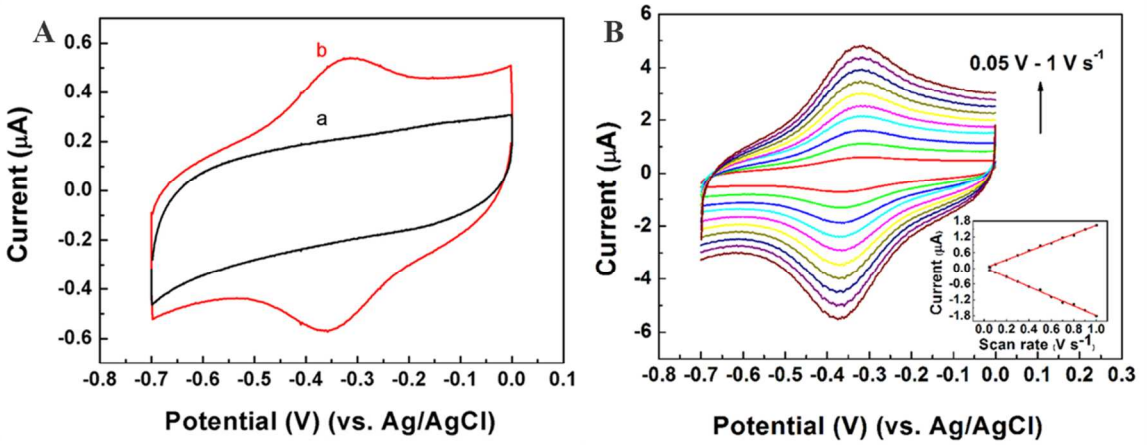


Figure 3. (A) CVs of the (a) Fmoc-FF hydrogel/GCE and (b) HRP/Fmoc-FF hydrogel/GCE in 0.1 M PBS. (B) CVs of the HRP/Fmoc-FF hydrogel/GCE in 0.1 M PBS at different scan rates. Peak current with scan rate is shown in the plot of inset.

Analytical performance of HRP/Fmoc-FF hydrogel/GCE for H_2O_2

First-generation enzyme biosensors are susceptible to the influence of oxygen, which affects their stability. Second-generation biosensors suffer from drawbacks from the diffusion barrier of the mediator, affecting their specificity. Third-generation enzyme biosensors are characterized by their inherent simplicity and high selectivity. [30,31] The Fmoc-FF hydrogel can offer advantageous environment for maintaining HRP activity and realizing direct electron transfer of HRP, as mentioned above. Thus, the data obtained above suggest the potential of a third generation of H_2O_2 sensor upon the HRP/Fmoc-FF hydrogel/GCE. Figure 4A showed CVs of the HRP/Fmoc-FF hydrogel/GCE in 0.1 M PBS, recorded in the modified electrode with and without absence and presence of H_2O_2. It can be seen that the cathodic peak increased and the anodic peak current decreased after the addition of H_2O_2. No response was observed at the Fmoc-FF hydrogel/GCE surface (Figure S1). These results indicated that HRP encapsulated in the Fmoc-FF hydrogel exhibited good catalytic activity toward H_2O_2 reduction.

The HRP/Fmoc-FF hydrogel/GCE as a third-generation biosensor for the detection of H_2O_2 was further researched by amperometry. Figure 4B displayed a typical current-time plot for the biosensor, with continuous additions of H_2O_2 to N_2-saturated 0.1 M PBS. When H_2O_2 was added into the stirred PBS, the current response quickly increased. The response time was less than 5 seconds, indicating that the biosensor has a fast response. The linear range of the biosensor was 1.0×10^{-7} to 6.0×10^{-5} M, and the correlation coefficient is 0.995 (Figure 4C). The detection limit for H_2O_2 was 18 nM, based upon S/N of 3. The current H_2O_2 biosensor is compared with reported HRP-based

direct electrochemical sensors in Table S1, which shows that the current HRP/Fmoc-FF hydrogel/GCE had a lower detection limit. The sensitivity (0.29 μA μM^{-1} cm^{-2}) of this biosensor was lower than that of HRP immobilized on inorganic nanomaterials which have high specific surface area and good conductivity.[32] However, the sensitivity of the current biosensor was higher than those using organic hydrogels (e.g. chitosan, polypyrrole and poly(thionine) films) for HRP supports, because of their weak conductivity.[33-35] The fast and sensitive response of the current HRP/Fmoc-FF hydrogel/GCE was attributed to its porous 3D networks. This is advantageous to the the HRP immobilization, maintained the high activity of the enzyme, as well as facilitated the diffusion of the substrate to the entrapped enzyme. Thus, the HRP/Fmoc-FF hydrogel/GCE has potential in applications that detect H_2O_2 released from living cells.

The selectivity of the biosensor for H_2O_2 was investigated by adding interfering substances for instance dopamine, ascorbic acid, uric acid, citric acid, glucose, and tyrosine, and also OH•, which can be produced when cells are stimulated using PMA. The amperometric responses to these interferents at –0.35 V are shown in Figure 4D. No obvious interference in H_2O_2 detection was observed at the applied potential, suggesting that the biosensor has satisfactory selectivity for the detection of H_2O_2. The stability and reproducibility were also evaluated. Figure S2 showed the voltammetric response nearly no change after scanning for 100 cycles. This indicated that HRP was immobilized in a stable manner within the Fmoc-FF hydrogel. Moreover, the HRP encapsulated in the Fmoc-FF hydrogel retained its activity and electrocatalytic performance for H_2O_2 over a wide pH range (Figure S3). When the HRP/Fmoc-FF hydrogel/GCE was stored at 4 °C

and used for the detection of 10 μM H_2O_2 every 2 days, the response of the current maintained more than 85% of its original value over 2 weeks (Figure S4). In addition, determining five times using a single-enzyme electrode, the relative standard deviation (RSD) was 2.3%. Five HRP/Fmoc-FF hydrogel/GCEs prepared independently were used to test 10 μM H_2O_2, and collectively yielded a RSD of 4.8%. Thus, the stability and reproducibility of the current biosensor were satisfactory.

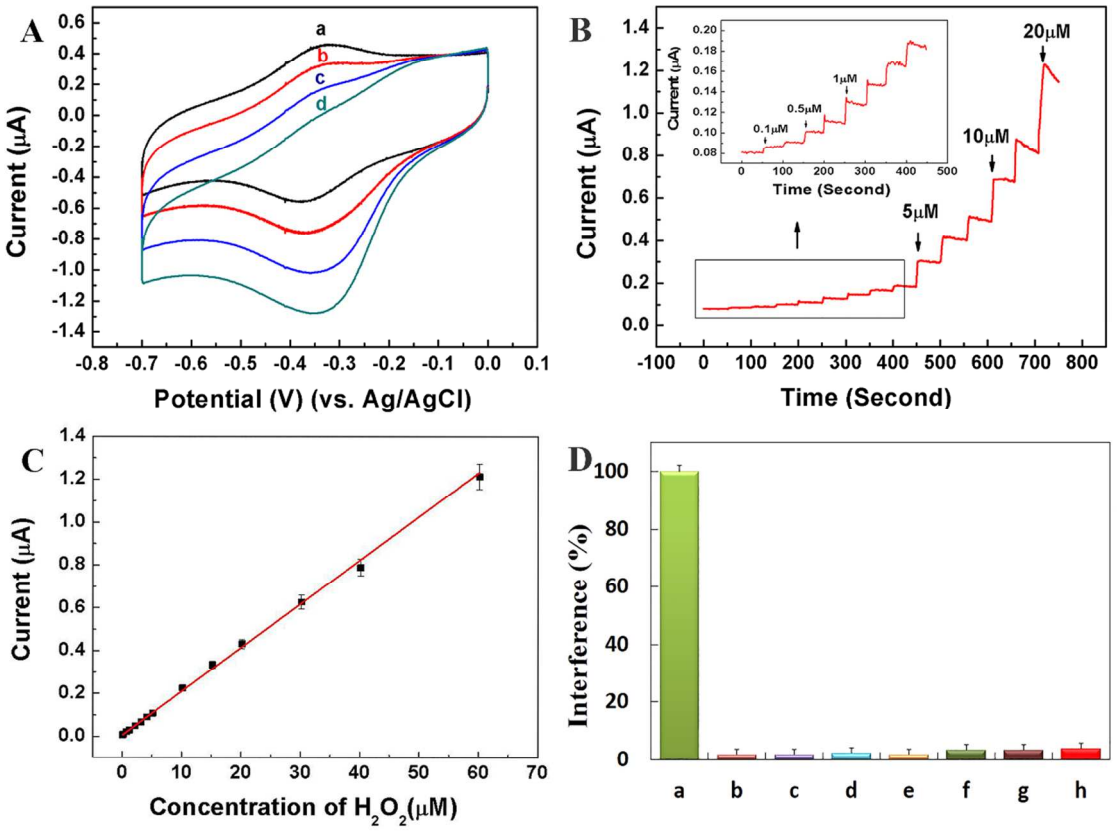


Figure 4. (A) CVs of the HRP/Fmoc-FF hydrogel/GCE in the presence of (a) 0, (b) 10, (c) 30, and (d) 50 μM H_2O_2 in 0.1 M PBS. (B) Current-time response of the HRP/Fmoc-FF hydrogel/GCE to the continuous addition of H_2O_2 in stirred 0.1 M PBS. (C) Plot of steady-state current vs. H_2O_2 concentration. (D) Selectivity obtained toward the addition of (a) H_2O_2, (b) dopamine, (c) ascorbic acid, (d) uric acid, (e) citric acid, (f) glucose, (g) tyrosine, and (h) OH• (10 μM for a–g).

In situ detection of H_2O_2 released from living cells

The above results proved the good analytical performance of this sensor, and its excellent biocompatibility as well as similar dimensions of the peptide hydrogel

nanofibers to the ECM. Thus, in situ detection of H_2O_2 released from HeLa cells was explored. The surface of the HRP/Fmoc-FF hydrogel/GCE were first used to immobilize HeLa cells. Figure 5A showed round HeLa cells in the surface of the HRP/Fmoc-FF hydrogel/GCE, and some small pseudopodia of HeLa cells were clearly observed in the inset of Figure 5A. This indicated that the HeLa cells could be adhered on the surface of the HRP/Fmoc-FF hydrogel/GCE. Further evidence was obtained from microscopy images of adhered cells and MTT assays. Figure S5 shows that cells were adhered to, and distributed about, the hydrogel surface in a fairly uniform manner.

Cell responses upon the addition of PMA which can trigger H_2O_2 production in human cells) were recorded, as shown in Figure 5B. An obvious increase in cathodic current was seen once the injection of PMA. The instant current response produced because of the rapid cellular secretion of H_2O_2, upon stimulation by PMA. The subsequent consumption of H_2O_2 from its reduction by HRP gradually decreased the peak current (curve a of Figure 5B). [36,37] As a control experiment, the same amount of PMA was injected into electrodes in the absence of HeLa cells. The resulting amperometric response showed negligible current increase (curve b of Figure 5B). This suggested that the increase in current resulted from the PMA-induced H_2O_2 released from cultured cells, which was effectively catalyzed by the HRP/Fmoc-FF hydrogel/GCE. The average number of extracellular H_2O_2 molecules released per cell (N_o) was calculated, according to a previously reported method.[2] For a current response of 109.7 nA (determined from curve a of Figure 5B), an electrode surface area of 7.07 mm^2, a sensitivity of 0.29 μA μM^{-1} cm^{-2}, a cell density of 4243 mm^{-2}, and an electrolyte volume of 1 mL, the N_o was

calculated to be about 10^{11} for 17 s (the detailed process is given in the Supporting Information). This value agrees well with a reported N_o value of 10^{11} for H_2O_2 released from MCF-7 cells on a layered graphene-artificial peroxidase-protein nanostructure.[2] This indicated that the current biosensor could be used to quantify the flux of H_2O_2 released from cells. The influence of PMA concentration for the release of H_2O_2 was also researched. The current response of H_2O_2 increased with the increase of the concentration of PMA as shown in Figure S4. This indicated that the observed signal was dependent on PMA concentration (Figure S6).

Figure S7 shows the responses of the HRP/Fmoc-FF hydrogel/GCE to HeLa cells in a conventional procedure, in which the H_2O_2 sensor electrode was physically located near living cell lines in solutions.[38,39] The measured amount of H_2O_2 was 1.3×10^{-15} mol per cell, which agreed well with reported values (2.1×10^{-15}, 2.95×10^{-15} mol, 1.05×10^{-16} mol per cell).[40,37,41] The measured amount of H_2O_2 was much lower than that obtained by cells directly adhered on the HRP/Fmoc-FF hydrogel/GCE (converting mole number, 1.7×10^{-13} mol per cell). The superior performance of the in situ monitoring of H_2O_2 was attributed to the smart biointerface consisting of living cells adhered to the hydrogel. The hydrogel suppressed the diffusion of H_2O_2 released from living cells, effectively accumulating the H_2O_2. This avoided any loss in sensitivity. The combined cell culture-enzyme design of the biosensor resulted in close proximity between the enzyme and release site. This enhanced the sensitivity because of the highly active and sensitive enzyme. The present design has the potential to detect molecules that are highly unstable and easily decayed. It also has potential in high-sensitivity single cell monitoring.

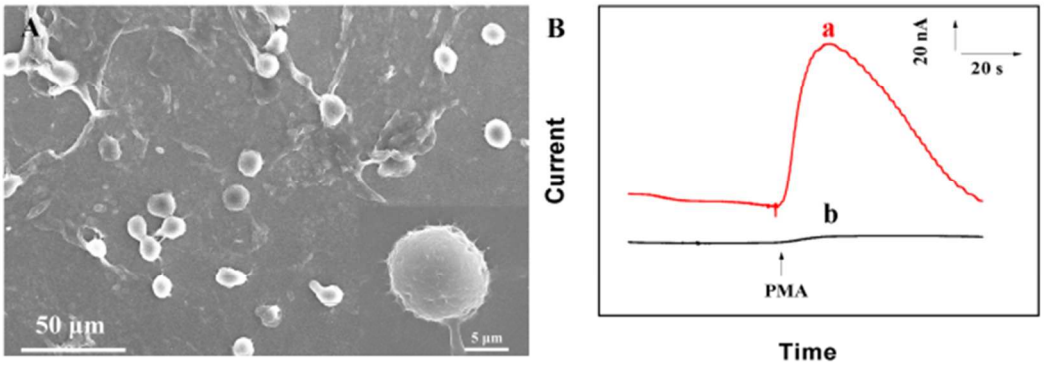


Figure 5. (A) SEM image of HeLa cells adhered to the HRP/Fmoc-FF hydrogel/GCE. The inset in (A) shows a high-magnification view. (B) Amperometric responses of the HRP/Fmoc-FF hydrogel/GCE upon the addition of PMA (1 μg mL^{-1}) to 0.1 M PBS with (a) and without (b) HeLa cells (3×10^4 cells) on the surface of the modified electrode.

CONCLUSIONS

We fabricated a smart biointerface using a Fmoc-FF peptide hydrogel, and applied it in enzyme encapsulation and cell adhesion. A combined cell adhesion-electrochemical biosensing platform based on the biointerface was obtained. The Fmoc-FF hydrogel exhibited good biocompatibility, a microporous structure, and supramolecular π-stacking along its backbone. This resulted in the immobilized HRP retaining its bioactivity, and realizing the electron transfer. Therefore fabricating a third-generation electrochemical biosensor for detecting H_2O_2, on the basic of the HRP/Fmoc-FF hydrogel/GCE. It exhibited a fast response, good selectivity, and reproducibility. HeLa cells could be adhered to the surface of the HRP/Fmoc-FF hydrogel/GCE, because of the good cell

adhesion, dimensional compatibility, and favorable mechanical stability of the Fmoc-FF hydrogel. The in situ quantitative and selective detection of H_2O_2 released from HeLa cells was realized along with high sensitivity. The present work provides a platform for the real-time detection of intracellular signal biological molecules. Various cells with recognition molecules such as DNA, proteins, and peptides can potentially be integrated into the hydrogel network in the process of the self-assembly of peptide. This hydrogel is low-cost, easily prepared, and environmentally friendly. The current platform has potential in applications involving cellular functions, drug discovery, and pathological investigations.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website. Fmoc-FF hydrogel/GCE response to H_2O_2, the influence of solution pH of biosensor, stability of biosensor, microscopy images of adhered cells, MTT assays, the effect of PMA concentration on the release of H_2O_2, and the responses of HRP/Fmoc-FF hydrogel/GCE to HeLa cells in solution.

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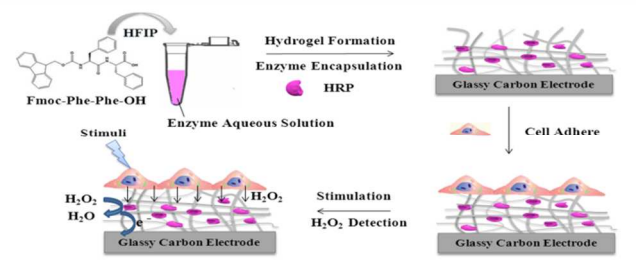
HFIP
Fmoc-Phe-Phe-OH
Hydrogel Formation
Enzyme Encapsulation
HRP
Enzyme Aqueous Solution
Glassy Carbon Electrode
Cell Adhere
Stimuli
H_2O_2
H_2O
H_2O_2
e
Glassy Carbon Electrode
Stimulation
H_2O_2 Detection
Glassy Carbon Electrode