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A supersensitive biosensor based on MoS_2 nanosheets array for the real-time detection of H_2O_2 secreted from living cells

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The fast and accurate real-time monitor of hydrogen peroxide (H_2O_2) secreted from the living cells plays a critical role for clinical diagnosis and management. Herein, we report a low-cost and self-supported MoS_2 nanosheets array towards non-enzymatic eletrochemical H_2O_2 detection. Under the optimal test conditions, such MoS_2 electrode presents extremely promising electrocatalytic performance with a low detection limit of 1.0 μM (S/N= 3) and an excellent sensitivity of 5.3 mA mM^{-1} cm^{-2}. Furthermore, the detection of the trace amount of H_2O_2 secreted from live A549 cancer cells was successfully performed on this biosensor.

Highly sensitive and effective determination of hydrogen peroxide (H_2O_2) concentration is of utmost importance in the fields of textile industries, bioanalysis, pharmaceutical security as well as environmental protection.[1,2] Peculiarly, H_2O_2 is a critical small molecule involving in immune responses, cell growth and signal transduction in different biological tissues.[3-7] Intracellular H_2O_2 concentration level is a key physiological parameter for early primary cancer screening and diagnostics, which opens a new horizon for reducing the mortality rate of cancer patient.[8] As a consequence, developing accurate and convenient analytical technologies for real-time H_2O_2 sensing is necessary and urgent in vitro and in vivo. Compared to conventional techniques for H_2O_2 detection including phosphorescence, fluorescence/luminescence,[9-14] chromatography,[15-17] and spectrophotometry,[18] much efforts have been devoted to designing and developing electrochemical sensing methods due to its advantages consisting of simple operation, desirable selectivity and quick responsiveness. At present, the enzyme-based electrochemical biosensors have gained ever-increasing attention in terms of its high sensitivity and superior specificity. Nevertheless, most of the reported enzymatic sensors still suffer from intrinsic problems containing complicated preparation procedures, low durability, and high cost, which has severely influenced the efficiency and limited their application in practice.[19] In sharp contrast, the enzyme-free electrochemical biosensors based on nanostructured materials present long-term stability, wide application range and quick responsiveness.[20-21] Although the noble metal-based biosensors possess excellent electrochemical detection performance, the limited storage and high cost hinder its widespread use.[22-25] Thus, it is highly attractive to build earth-abundant elements-based biosensors to realize supersensitive detection.

In previous work, MoS_2 has been reported as a high-performance catalyst for the hydrogen evolution reaction and oxygen reduction reaction and presented the promising potential for the construction of electrocatalytical biosensors due to its excellent electrocatalytical properties.[26,27] Recently, MoS_2 nanoparticles and interlayer-expanded MoS_2 were designed as the electrochemical biosensors for H_2O_2 detection with a low detection limit.[28,29] Although impressive H_2O_2 detection performance has been achieved, a prime challenge still lies in the sensitivity. The construction of self-supported nanoarrays has been proposed as an effective strategy to increase the electrochemical performance of nanomaterials by virtue of increasing catalytic sites, enhancing electronic conductivity and contact area.[30,31] Thus, we anticipate the electrochemical sensing ability of MoS_2 can be further enhanced by constructing self-supported nanoarrays.

In this communication, we report the synthesis of MoS_2 nanosheets array grown on carbon cloth (MoS_2/CC) for supersensitive monitoring of H_2O_2. Furthermore, the trace amount of H_2O_2 that produced from live cells was successfully recorded on the basis of this MoS_2/CC biosensor. Satisfactorily, the proposed MoS_2/CC electrochemical biosensor presents super sensing performance with a low detection limit of 1.0 μM (S/N = 3) and an excellent sensitivity of 5.3 mA mM^{-1} cm^{-2}. This work offers considerably significant guidance for designing biosensors in the fields of electrochemical sensing.

Fig. 1a shows the X-ray diffraction (XRD) pattern for the resulting MoS_2/CC, several diffraction peaks of MoS_2/CC can be assigned to the MoS_2 phase (JCPDS No. 37-1492). Additional, the diffraction peak at 26.2° can be indexed to bare CC substrate. No other diffraction peaks can be observed, indicating the high purity of the MoS_2/CC sample. The scanning electron microscopy (SEM) image (Fig. 1b) indicates the full coverage of bare CC (inset) with MoS_2 nanosheets. The magnified SEM image (Fig. 1c) further reveals that the MoS_2 has a typical sheet-like structure. The high-resolution TEM (HRTEM, Fig. 1e) image taken from single nanosheet (Fig. 1d)

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exhibits a well-resolved lattice fringe with interlayer spacing of 0.164 nm, corresponding to the (106) plane of the hexagonal MoS_2 crystals. The selected area electron diffraction (SAED) pattern (Fig. S1) shows three diffraction rings indexed to the (004), (101) and (106) planes of the MoS_2 phase. Additional, the energy-dispersive X-ray (EDS) spectrum of MoS_2/CC indicates the coexistence of Mo and S elements with an atomic ratio of 0.35 : 0.65 (Fig. S2). And the EDS elemental mapping analysis further confirms the uniform distribution of Mo and S elements within the whole nanoarrays (Fig. 1f). Furthermore, the X-ray photoelectron spectroscopy (XPS) survey spectrum of MoS_2/CC also confirms the presence of Mo and S elements (Fig. S3a). In Fig. S3b, the binding energies (BEs) at 232.2 and 229.1 eV are correspond to Mo $3d_{3/2}$ and Mo $3d_{5/2}$ in the Mo 3d region, indicating the existence of Mo^{2+}.[32] The S 2s peaks located at 226.7 eV can also be seen in the Mo region. In Fig. S3c, the S 2p spectrum shows two obvious peaks with BEs of 232.5 and 235.3 eV, attributing to S $2p_{3/2}$ and S $2p_{1/2}$ respectively, indicating the presence of S^{2-}.[33] Such above observations collectively support the successful synthesis of MoS_2 nanosheets array.

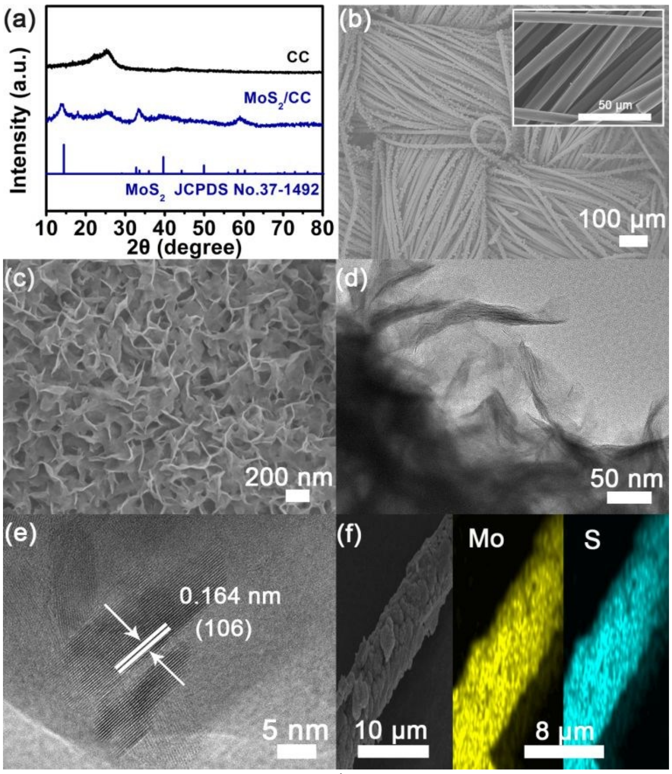

Fig. 1. (a) XRD patterns of CC and MoS_2/CC. (b, c) SEM images of CC (inset) and MoS_2/CC with different magnifications. TEM (d) and high-resolution TEM (e) images of MoS_2 nanosheets. (f) EDS elemental mapping images of Mo and S for MoS_2/CC.

The cyclic voltammetry (CV) is preferably applied to reveal the catalytic activity of designed biosensor for H_2O_2 reduction. In Fig. 2a, the bare CC (curve 1 and 2) displays the nearly similar current density in the absence and presence of 1 mM H_2O_2 in 0.1 M PBS, indicating that bare CC electrode is electrochemical inert for the detection of H_2O_2. On the contrary, the reduction current density of the MoS_2/CC electrode (curve 3 and 4) is notable improved with a ~200% increase after adding 1 mM H_2O_2, suggesting that the obtained MoS_2/CC electrode is feasible for H_2O_2 detection. As plotted in Fig. 2b, the cathode-current densities are accordingly enhanced with increasing of H_2O_2 concentration from 0 to 3 mM, which revealed that MoS_2/CC is suitable for construction of the electrochemical biosensors due to the efficient electrocatalytic performance. According to the former research,[34] we hypothesize that the proposed biosensor is based on the following detection mechanism, but possibly through other mechanisms. MoS_2/CC as the good electron conductors facilitates the electron transfer from MoS_2/CC electrode to reduce H_2O_2.

$$MoS_{2\,(Ox)} + e^- \leftrightarrows MoS_{2\,(Red)} \quad (1)$$

$$MoS_{2\,(Red)} + 2\,H_2O_2 \leftrightarrows MoS_{2\,(Ox)} + 2\,H_2O + O_2 \quad (2)$$

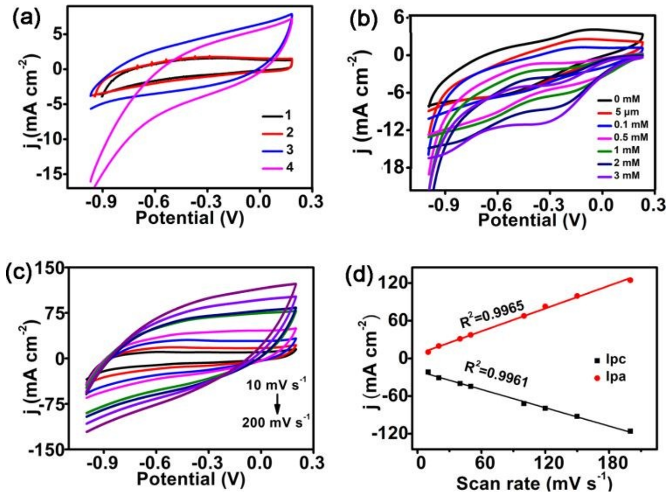

Fig. 2. (a) CVs of bare CC (curve 1 and 2) and MoS_2/CC (curve 3 and 4) in the absence and presence of 1 mM H_2O_2 in 0.1 M PBS (pH =7.4) (scan rate: 50 mV s^{-1}). (b) CVs of MoS_2/CC in 0.1 M PBS with varied H_2O_2 concentrations. (c) CVs of MoS_2/CC in 0.1 M PBS containing 1 mM H_2O_2 at scan rates from 5 mV s^{-1} to 200 mV s^{-1}. (d) The plots of current density of H_2O_2 vs. the scan rates.

The CV curves of MoS_2/CC were collected within the range between 10~ 200 mV s^{-1} in 0.1 M PBS containing 1 mM H_2O_2 (Fig. 2c). As the results shown in Fig. 2c, the current densities at the cathode and anode augment continuously upon increasing the scan rate on the CV scanning. Obviously, both anodic and cathodic current densities is proportional to scan rates (Fig. 2d), indicating that the process of electrochemical reduction follows a typical absorption control mechanism.[35,36]

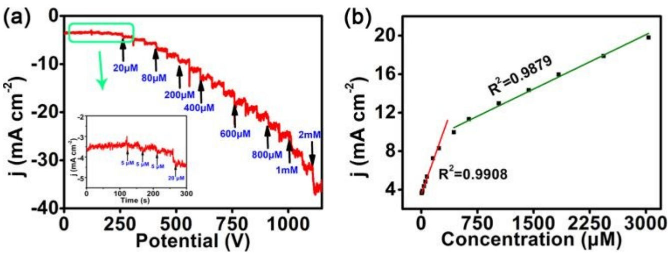

Fig. 3 (a) Amperometric response of MoS_2/CC for the successive addition of H_2O_2 in 0.1 M PBS (pH = 7.4). Applied potential: -0.5 V. The inset shows the amperometric response of H_2O_2 at low concentration. (b) The dependence of current density of H_2O_2 vs. concentration.

Before electrochemical sensing testing, it is important to acquire the most appropriate reduction potential by chronoamperometry. The optimum applied potential for the electrochemical sensing requires to follow two requirements: i) a larger current response, ii) a lower signal-to-noise ratio. Fig. S4 shows the amperometric current response of the biosensor with the successively addition of 1 mM H_2O_2 into the stirring PBS under different potentials. According to current-time (i-t) curves, -0.5 V was chosen as best potential in subsequent experiment. After that, the current response of MoS_2/CC for H_2O_2 sensing with the successive addition of H_2O_2 into the electrolyte solution was performed under -0.5 V to assess the sensitivity for electrochemical detection. As shown in Fig. 3a, the fast current response can be observed with the increased

H_2O_2 concentration and the current platforms was steady within 3s. The inset of Fig. 3a shows the amplified amperometric response of H_2O_2 at low concentration region. Accordingly, Fig. 3b presents the linear functional relationship of current density vs. H_2O_2 concentration at a range of 5.0 to 3.0 × 10^3 μM. There are two linear calibration plots corresponding to the low (5.0–2.35 × 10^2 μM) and medium (4.35 × 10^2–3.0 × 10^3 μM) concentration ranges. They are in good fit with the regression equations of j = 3.53 + 0.0218 × $C_{H_2O_2}$ (R^2 = 0.9908) and j = 8.81 + 0.0038 × $C_{H_2O_2}$ (R^2 = 0.9879), respectively. Especially, the sensitivity in the two concentration ranges are as high as 5.3 and 3.6 mA mM^{-1} cm^{-2}, respectively. Furthermore, the detection limit is estimated to be 1.0 μM (S/N = 3), suggesting that the proposed biosensor has superior to a number of recently developed electrochemical H_2O_2 sensors (Table S1).

Lung cancer is one of the most common malignancies worldwide and poses a serious security threat to people's life and health.[37] Hence, we chose A549 cells as model cells for check the possibility of practical application of MoS_2/CC to detect H_2O_2 in this work。Phorbol-12-myristate-13-acetate (PMA) is used to stimulate the production of H_2O_2 in cells.[38,39] As depicted in Fig. 4, the amperometric current density response increased apparently upon adding PMA into 20 mL of PBS (pH = 7.4) containing 4×10^7 cells, while no signal could be observed if cells were not treated with PMA. This phenomenon proves that the recorded current originates from the reduction of the H_2O_2, releasing from A549 cells upon being stimulated. In summary, the developed MoS_2/CC biosensor is suitable for real-time monitoring of extracellular H_2O_2 for clinical research.

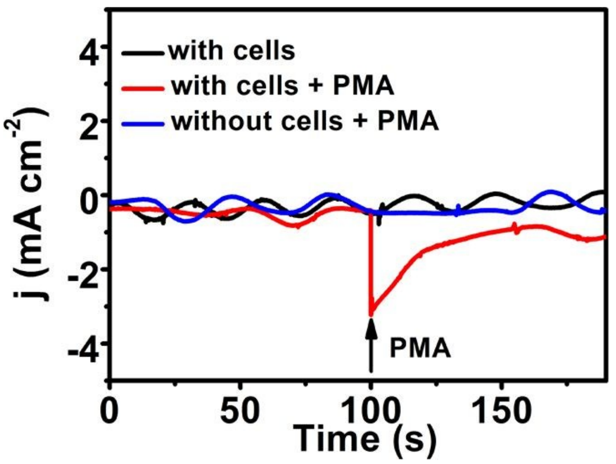

Fig. 4 Amperometric responses of MoS_2/CC to the stimulations in 0.1 M PBS (pH = 7.4) with and without A549 cells. Applied potential: -0.5 V.

Considering the practical application, the selectivity and stability are equally crucial as major factor of the analytical properties. The current changed with the injection of some interfering substances (such as AA, UA, DA, NaCl, L-Cys) and H_2O_2 into PBS successively at -0.5 V are compared to assess the specificity of the proposed biosensor. As presented in Fig. S5, the current responds obviously towards H_2O_2, while the response is negligible to the variation in the concentration of other interferences. The anti-interference test suggests the MoS_2/CC electrode possesses acceptable selectivity for H_2O_2. The stability of the MoS_2/CC biosensor is evaluated by recording 20 times successive CV responses of 1.0 mM H_2O_2. As shown in Fig. S6, the current density only presents slight loss during multiple cycles in 0.1 M PBS containing 1.0 mM H_2O_2, suggesting the satisfying stability of the proposed biosensor.

In conclusion, the MoS_2 nanoarrays directly grown on carbon cloth was prepared and explored its electrochemical sensing ability towards H_2O_2. The obtained MoS_2/CC exhibits attractive electrocatalytic activity with low detection limit and excellent sensitivity and selectivity due to its abundant surface area and low charge transfer resistance. More significantly, a non-enzymatic H_2O_2 biosensor based the as-prepared MoS_2/CC has extremely high sensitivity in determination of trace H_2O_2 amount released from A549 cells. This study opens an avenue for the application of self-supported nanoarrays in the fields of electrochemical sensing.

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