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A porous hollow fiber sensor for detection of cellular hydrogen peroxide release based on cell-in-lumen configuration

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ABSTRACT

The rapid and accurate detection of cellular hydrogen peroxide (H_2O_2) is of great significance in cell monitoring and drug screening. We have developed a porous hollow fiber sensor with a cell-in-lumen configuration to detect H_2O_2 released by cells in real-time. In contrast to the conventional two-dimensional electrode structure, the sensing unit was located at the outer surface of the hollow fiber, while cells grew in the lumen, and the released H_2O_2 can diffuse through the porous wall of the fiber. The sensing layer wrapped outside the porous hollow fiber can capture the released H_2O_2 in a flow-through mode, resulting in a high sensitivity to the nanomolar level. In short-term monitoring of cell respiratory burst, the current response of the cell-in-lumen sensing configuration was six times greater than that of a conventional cell-out-electrode configuration. The porous wall also separated the cells and electrodes, minimizing the impact of electrical field on the cells. The high sensitivity and stability of detection ensured the cell-in-lumen configuration could be used to monitor the cellular H_2O_2 gradual release process induced by anticancer drugs. Such in-situ monitoring of the cellular release of H_2O_2 provides a new in-vitro drug screening platform for personalized medicine and cancer therapy.

1. Introduction

Reactive oxygen species (ROS) act as molecule mediators in cell signaling, and play an essential role in biological processes in normal and cancer cells [1,2]. Hydrogen peroxide (H_2O_2) is a major ROS in cells that have been found highly related to signal transduction pathways and cell fate decisions [3–6]. H_2O_2 is generated from the dismutation of the superoxide anion radical in response to various stimuli [7,8]. H_2O_2 exhibits either pro- or anti-apoptotic activities [9]. Excessive production of H_2O_2 causes oxidative stress and induces malignant transformation of cells [10]. Cancer cells have been found to be prone to apoptosis induced under high concentration of H_2O_2. Thus, triggering cellular H_2O_2 production is a mechanism used by many chemotherapeutics [11,12]. Given the significance of cellular H_2O_2 in cell pharmacology and pathophysiology, accurate and reliable measurement of cellular H_2O_2 need to be established.

The development of cellular H_2O_2 sensor is restricted by many factors, including small cell size, low concentration of cellular H_2O_2 and interferences in the culture medium [13,14]. Methods for detecting H_2O_2 include fluorescence, chemiluminescence, colorimetry, chromatography and electrochemistry [15–18]. The release of H_2O_2 from cells is a time-related accumulation process. End-point sampling and interval sampling methods could not continuously monitor the concentration change in real-time. Enzyme-based electrochemical sensing is efficient for in-situ and continuously monitoring of H_2O_2 because of its high sensitivity, rapid response and selectivity [19,20]. Due to the low concentration of H_2O_2 generated by cells in response to external stimuli, new structures and novel materials have been introduced to increase sensitivity and stability of the electrochemical sensor.

The basic configurations of H_2O_2 sensors can be summarized into two groups. One approach is to grow cells directly adhered to the electrode. The near-by electrode could effectively capture and monitor the H_2O_2 released from cells at low concentrations. Biocompatible materials in two-dimensional or three-dimensional structures have been applied as electrode interfaces in this approach [21,22]. But direct cell adhesion to the electrode induces electrical stimulation and alters cell metabolic activities in long-term monitoring. The other approach is immersing the sensor electrode in the cell culture environment by measuring the H_2O_2 concentration change in the bulk medium. Different nanomaterials such as graphene, carbon nanotubes and metal

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nanoparticles are used to modify the electrode to improve its performance [23,24]. The sensitivity of this approach depends on the cell density, cell suspension status and liquid diffusion, making subtle dynamic changes difficult to detect. Therefore, most electrochemical sensors focus on detecting short-term cellular H_2O_2, while there are few works on long-term monitoring of low-concentration H_2O_2 release [13,18,19,21,25].

The hollow fiber system has been widely used as cell culture platforms in the biomanufacturing and biopharmaceutical industries due to its long-term stability and high cell density [26,27]. Inspired by the combination of living cells and hollow fiber, we developed a flow-through sensing strategy based on cell-in-lumen configuration. The cells grow on the inner surface of a porous hollow fiber (PHF), while sensing layers including multi-walled carbon nanotubes, gold nanoparticles (AuNPs) and enzymes were immobilized on the outer surface of the PHF. The porous structure provides liquid exchange channels so that the cell released H_2O_2 can reach the sensing layer in a short distance. The cell-in-lumen PHF structure ensured that most of the H_2O_2 released from cells first flowed through the electrode layer and then diffused into the medium. This structural feature enables the detection of low concentration down to the nanomolar level. The porous structure also separated the sensing layers and the cells, minimizing the electric field interference. Experimental results also showed that the sensor was able to continuously monitor the trace amount of H_2O_2 released from the cells. The unique PHF structure, combined with enzyme-nanoparticles selective catalysis, allows this platform to be used in real-time tracking of reactive molecular in cell culture, toxicity screening and drug discovery.

2. Experimental section

2.1. Material and instruments

Horseradish Peroxidase (HRP), Polysulfone (PSF), Chloroauric acid ($HAuCl_4$), Polyvinylpyrrolidone (PVP), Polyethylene glycol (PEG), Dimethylacetamide (DMAC) were purchased from Sinopharm Chemical Reagent (Shanghai, China). Multi-walled carbon nanotubes (MWNT) dispersion (Diameter: 50 nm, Length: < 10 μm) were provided by Xfnano Materials Tech Co., Ltd. Dulbecco's modified eagle's medium (DMEM), Trypsin-EDTA (0.25 %) and phosphate buffer solution (PBS) were purchased from HyClone (Waltham, USA). Fetal bovine serum (FBS) was obtained from Gibco (USA). All aqueous solutions were prepared with Milli-Q water (18.2 MΩ cm, Millipore). Morphology of the PHF sensor was investigated by Hitachi SU-8000 field emission scanning electron microscope (SEM). The cyclic voltammetry (CV) and amperometric response (I-t) were recorded using CHI660 electrochemical workstation with a three-electrode system. Ag/AgCl and platinum wire were used as the reference electrode and the counter electrode. Fabricated PHF sensor was used as the working electrode. Fluorescence confocal images of cells on the fiber inner surface were captured by Olympus FV3000 confocal microscope.

2.2. Fabrication of hollow fiber

The H_2O_2 sensor is fabricated based on a Polysulfone (PSF) hollow fiber structure. PSF hollow fiber was prepared by a non-solvent induced phase separation method according to our group's previous work [28]. Briefly, 18 wt% PSF, 6 wt% PVP and 16 wt% PEG in DMAC solution was extruded from the spinning head to form PHF, which passed through coagulation baths to form a porous structure. After phase separation, the PHF was soaked in 15 % aqueous glycerol solutions for 24 h.

2.3. Preparation of PHF sensor

Fig. 1 illustrates the step-by-step procedure to construct the sensing layers on the outer surface of PHF. First, PHF was vertically immersed into the MWNT water dispersion (1 wt%) with one end sealed. When the other end was connected to a vacuum pump, the MWNT would be filtered to form a conductive mesh layer on the PHF surface, whose thickness was determined by the volume of MWNT dispersion filtered. Next, PHF was immersed in the 0.5 M H_2SO_4 solution with 0.35 mg/mL $HAuCl_4$. AuNPs were deposited on the MWNT layer by electrodeposition under -0.2 V constant potential. The modified PHF was soaked for 2 h in HRP aqueous solution (1 mg/mL), followed by 0.25 % glutaraldehyde for enzyme immobilization. The assembled electrode was rinsed with water to remove the unbound HRP and dried in air.

2.4. Cell culture and manipulation

In cell experiments, human lung cancer H1299 cell suspension was injected into the PHF. The PHF with cells was cultured in a CO_2 incubator at 37°C with high glucose DMEM containing 10 % FBS. For confocal microscope observation, H1299 cells in PHFs were incubated with DAPI staining solution (0.1 % Tween-20 and 1% BSA in PBS) for 30 min and rinsed with PBS extensively. For SEM characterization, H1299 cells were fixed in 4% formaldehyde for 20 min, followed by thorough washing. Cell viability in lumen was determined by adding 10 % (v/v) Alamar Blue (Biosciences) in cell culture media. After re-incubated at 37 °C, 5% CO_2 for four hours, Alamar Blue was reduced by living cells to fluorescent red, which was measured at 545 nm E_x/590 nm E_m by a Biotek Plate Reader. PHF with cells were washed with PBS and incubated in media again for further proliferation after each Alamar Blue test.

2.5. Detection of H_2O_2 released from H1299 cells

For H_2O_2 cell-in-lumen monitoring, one end of the PHF was sealed by paraffin, and 1×10^5 H1299 cells were injected into the PHF. Cell number was estimated by the Countess II cell counter. MWNT on the PHF served as a conductive layer was connected to the electrochemical workstation. Ag/AgCl and platinum wire as the reference electrode and counter electrode were inserted in the solution. Before Testing, the system was kept in a CO_2 incubator for 4 h to promote cell adhesion to the inner surface of PHF. The current response indicates H_2O_2 concentration change was recorded through the whole experiment by an electrochemical workstation at -0.4 V. Phorbol 12-myristate 13-acetate (PMA), formylmethionyl-leucyl-phenylalanine (fMLP) and Doxorubicin (DOX) were used to stimulate cells to generate H_2O_2. These small molecule drugs can penetrate into the lumen through the porous wall. H_2O_2 released from the cells diffuses through the porous structure and can be detected in electrochemical measurements.

The cell-out-electrode configuration was tested as a control group. Instead of injecting cells in fiber lumen, the same number of cells were applied outside the PHF in 10 mL culture medium. PHF sensor without cells inside was used as a working electrode and immersed in the medium to detect the changes of H_2O_2 concentration. In all experiments, the immersion depth of PHF was kept at 5 cm.

3. Results and discussion

3.1. Structure concept and design

Cell generated H_2O_2 is in extremely low concentration, but effectively functions in cell proliferation, differentiation, inflammation, circadian rhythm and apoptosis [7]. According to previous references, in a drug-induced respiratory burst, a single cell can generate 2×10^{-15} mol H_2O_2 [29]. In general, the H_2O_2 sensing electrode is inserted into the cell culture medium as a 'cell-out-electrode' configuration, and the cell-produced H_2O_2 randomly diffuses and contacts with the sensing electrode to generate electrochemical signals. Random diffusion in bulk medium impedes the sensitivity improvement of sensing. Based on these limitations, we designed and fabricated a hollow

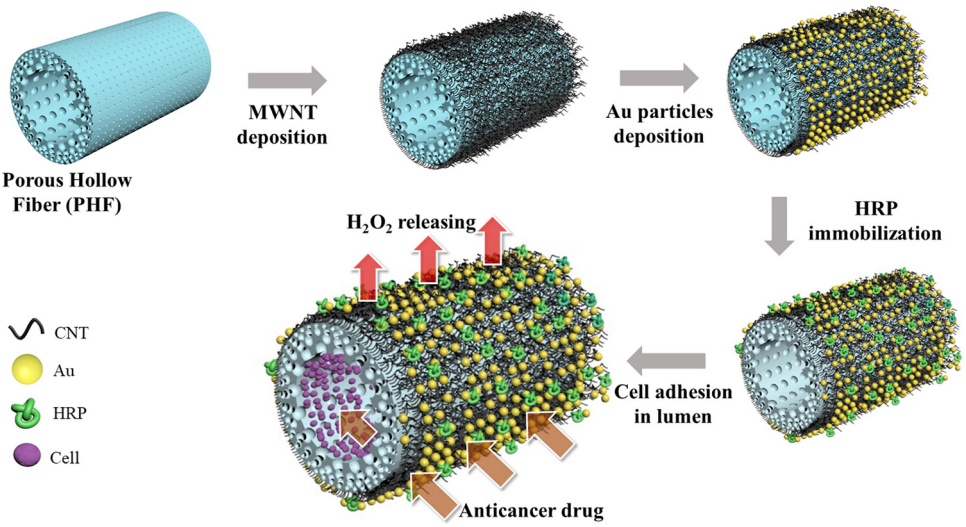

Fig. 1. Schematic illustration of the PHF electrode fabrication procedure and sensing principle.

fiber sensor (Fig. 1) to optimize the spatial relation between the sensor and the cells. A sensing layer was built on the outer surface of PHF, and the cells grew within the PHF lumen. The liquid can be diffused and exchanged via the porous structure, taking full advantage of the hollow fiber structure. In this cell-in-lumen configuration, the majority of cell-produced H_2O_2 flew through the wrapped sensing layer, and the detection sensitivity was much higher than the conventional cell-out-electrode configuration.

3.2. Morphological characterization

The morphology and structure of the PHF electrode were examined by SEM. Fig. 2A shows the cross-section image of a PHF with an outer diameter of 550 μm and an inner diameter of 400 μm. Magnified images of the outer surface and inner surface were shown in Fig. S1A & B. The pores gradually became smaller from the inner surface (> 1 μm) to the outer surface (< 100 nm). The inner surface served as a cell adhesion layer, and the porous structure promised fluid exchange through the interconnected pores. Pore diameter PHF can be further tuned from tens of nanometers to several micrometers by controlling phase separation parameters. MWNT were used to form a 3D conducting framework at the outer surface, as shown in Fig. 2B & C. Unlike other bulky conductive materials, the MWNT retained a loose connective structure suitable for fluid exchange. As shown in Fig. 2D, AuNPs homogeneously deposited on the MWNT layer, and the particle diameter is 110 ± 30 nm. EDX analysis in Fig. S1C & D also proved the uniform deposition of Au on the PHF outer surface. PHF permeation performance in terms of water flux is presented in Fig. S2. The hydrophilic flux was tested for 60 min in the distilled water, and the average flux value remained 2018 L/(m^2 h) at 60 min under 0.1 MPa. The water flux decreased to 1940 L/m^2 h when the MWNT layer was deposited on the outer surface. The water flux slightly decreased to 1856 L/m^2 h after AuNPs and HRP immobilization. After the sensing layer immobilization, the overall flux decreased within 10 %, which indicated liquid permeation of PHF could be maintained.

3.3. Electrochemical studies

The electrochemical sensing performance in Fig. 3A & B was measured by successive injection of different concentrations of H_2O_2 into PBS solution under -0.4 V working potential. Upon addition of H_2O_2, the amperometric response increased and achieved 95 % of the maximum value within 20 s. The results showed that the reduction current of H_2O_2 on the HRP-AuNPs-MWNT had a linear relation with H_2O_2 concentration in the range of 0.01–5 μM. The response to 10 nM H_2O_2 can be detected (inset of Fig. 3A), and the sensitivity was 90 nA/μM. Considering the signal-to-noise ratio of 3, the limit of detection (LOD) of the PHF sensor can reach 6 nM. To verify the effectiveness of the HRP and AuNPs deposition onto MWNT, cyclic voltammetry was utilized by cycling from -0.8 V to 0.2 V in PBS with different concentrations of H_2O_2, as shown in Fig. 3C. The reduction peak current increased with increasing the concentration of H_2O_2, suggesting a typical electrocatalytic HRP reduction process.

Taking into consideration a large number of electroactive species present in the medium, sensor selectivity is a critical requirement for applications in the cell culture environment. Ascorbic acid (AA), acetaminophen (AP), Dopamine (DA), Glutathione (GSH) and uric acid (UA) are the typical interferences for electrochemical biosensing. As shown in Fig. 3D, the addition of interfering molecules induced a small current change, which was less than 1% of the current change magnitude induced by H_2O_2. These results showed a sensitive and selective electrochemical property of the PHF electrode toward H_2O_2, indicating its promising potential for real-time monitoring H_2O_2 release from cells. In the sensor stability test, all electrodes were immersed in PBS solution at 20 °C for one week and tested activities every day. Fig. S3 showed the activity of the PHF sensor retained above 89 % of its initial activity after one week, indicating promising stability for long-term monitoring. Six PHF sensors were prepared under the same condition to verify the relative standard deviation (RSD). The RSD of sensing current (Fig. S5) to the addition of 100 nM and 200 nM H_2O_2 was 3.72 % and 4.08 %, which confirmed the reproductivity of the results.

3.4. Cells adhesion on the PHF

PHF can provide a stable platform for cell adhesion that enables long-term monitoring of cellular H_2O_2. The cells cultured on the inner surface of the PHF were examined through SEM and confocal microscope. H1299 cells were cultured in PHF for 24 h and viewed with SEM to verify the cell adhesion to the inner surface of PHF. As shown in Fig. 4A, H1299 cells adhered to the as-prepared PHF porous surface, and there were a large number of pores on fiber inner surface for H_2O_2 released from cells to pass through. The image showed that uniform fluorescence could be seen through the length of the fiber, indicating a uniform distribution of the cells within the fiber lumen. To further verify the distribution of cells on the inner surface of the PHF, we cut open the PHF along its longitudinal direction and observed the cell distribution with a confocal microscope. Fig. 4B showed a 3D volume rendering fluorescence image of the H1299 cells' distribution on the

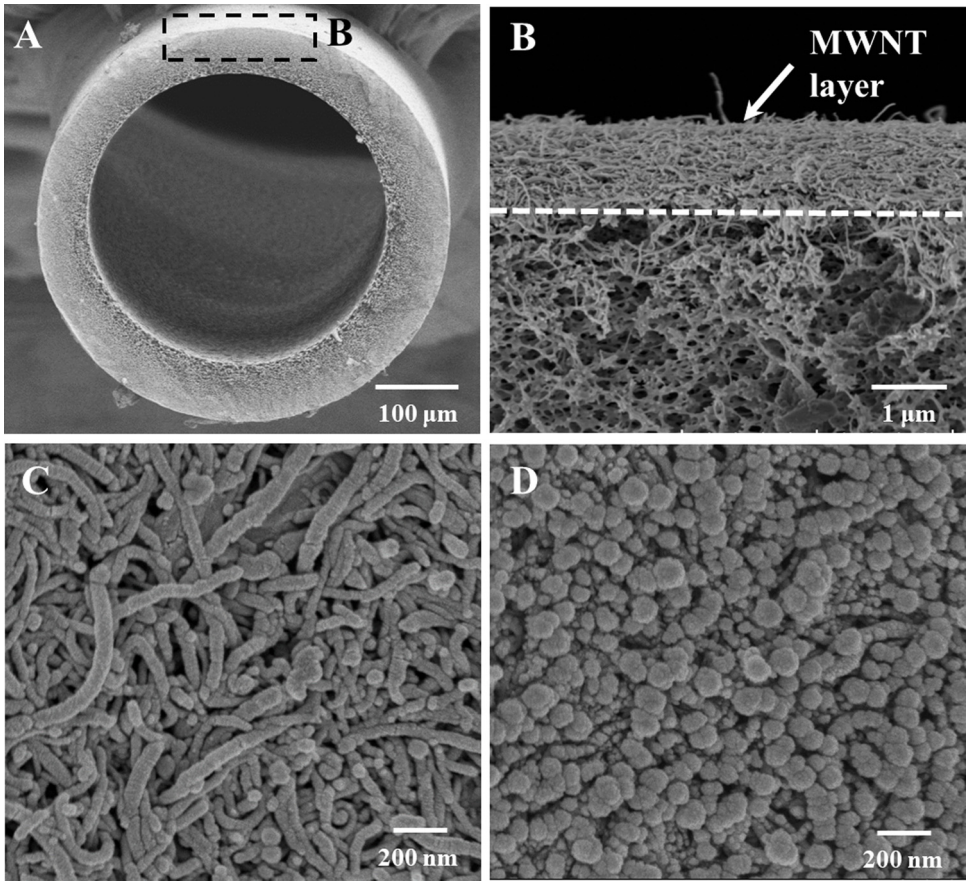

Fig. 2. (A) SEM images of the PHF cross-section structure. (B) Cross section view of MWNT layer deposited on the PHF outer surface. (C) Top view of the MWNT layer deposited PHF wall showing a gradient porous structure. (D) Top view of Au particles deposited on MWNT layer.

PHF inner surface. The cell fluorescence image showed a typical curved geometry structure indicating the close cell-PHF adhesion. We used Alamar Blue as a fluorescence indicator of cell viability in the lumen. The reagent was delivered to the cells across the hollow-fiber membrane, reduced by the cells and released from the cell back to the media again. Fig. 4C showed the development of fluorescence over time for 1×10^5 H1299 cells in lumen, indicating cell viability and proliferation in PHF over 24 h period.

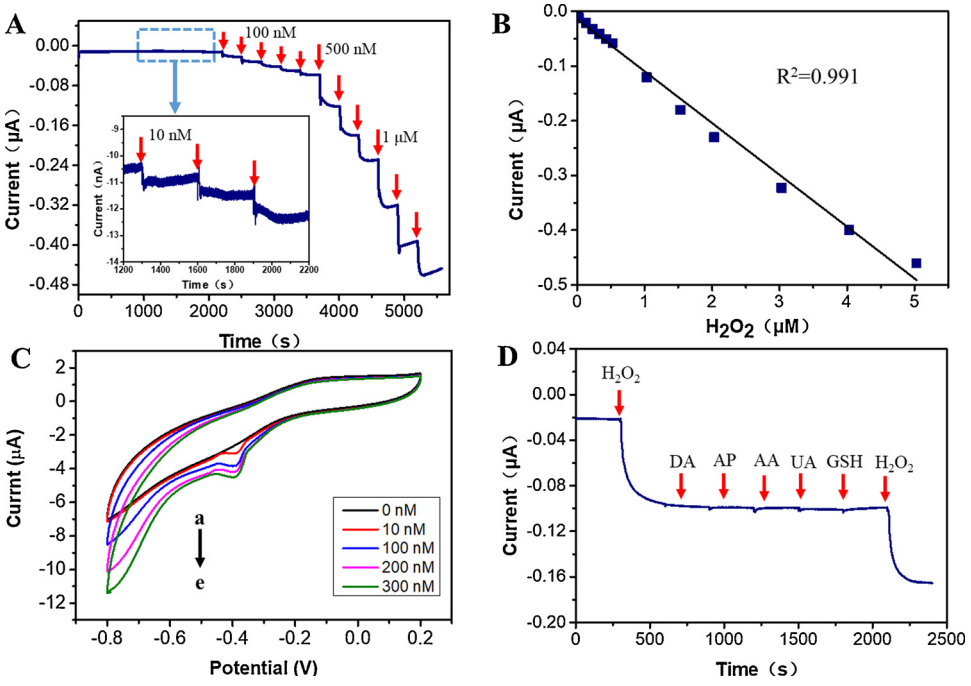

Fig. 3. (A) I-t curve of PHF sensor upon additions of H_2O_2 to PBS under -0.4 V working potential. Inset image: the detailed current response to 10 nM H_2O_2. (B) The calibration curve between sensor current and H_2O_2 concentration. (C) Cyclic voltammograms of PHF electrodes in PBS buffer containing different concentrations of H_2O_2: (a) 0, (b) 10, (c) 100, (d) 200, (e) 300 nM. Scan rate: 100 mV/s. (D) Amperometric responses of the PHF sensor to 0.5 μM of H_2O_2, DA, AP, AA, UA and GSH.

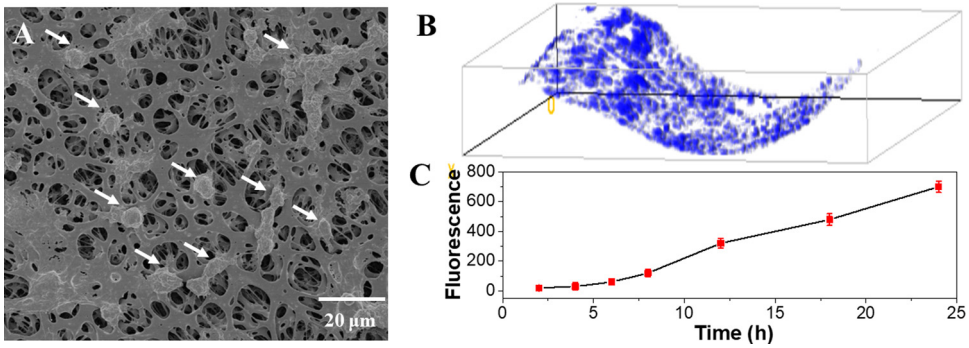

Fig. 4. (A) SEM of cells adhered on the PHF inner surface; (B) Volume view of fluorescence stained cells on the PHF inner surface. (C) Fluorescence intensity of H1299 cells in PHF lumen with different culture time.

3.5. Detecting of H_2O_2 released from cell respiratory burst

The dynamics of H_2O_2 releasing from lung cancer cell line H1299 were monitored by a three-electrode electrochemical system in the cell culture medium. One end of the PHF was sealed by paraffin, and 1×10^5 cells were injected into the lumen of PHF. The PHF loaded with cells was used as a working electrode, as shown in Fig. 5A. We also tested a cell-out-electrode configuration by immersing the PHF in the cell suspension medium. Like other commonly used cellular H_2O_2 detection setups, PHF was only used as an electrode in cell-out-electrode configuration, not as a cell adhesion substrate. Drugs, including PMA and fMLP, were capable of triggering cell respiratory burst to release a large amount of H_2O_2. In Fig. 5B, PMA was added to the culture medium outside the PHF and diffused into the fiber to stimulate the cells. The current increased obviously with the addition of 0.14 μg/mL PMA, and the maximum current 42 nA was reached in ten seconds. The maximum detected H_2O_2 concentration in the cell-in-lumen configuration was estimated as 467 nM based on the calibration tests in Fig. 3A. In the cell-out-electrode configuration, the same number of cells were dispersed in the solution outside the fiber, and PMA was added to the same 0.14 μg/mL concentration as the cell-in-lumen configuration. The sensor current increased by 7 nA, equivalent to a 77 nM increase in H_2O_2 concentration, which was over 80 % lower than the detection value in the cell-in-lumen configuration. In the cell-out-electrode configuration, most of the released H_2O_2 diffused or decomposed in bulk medium, making less H_2O_2 to react on the sensor electrode.

We further tested another commonly used cell-stimulating drug fMLP in the same experimental setups. In Fig. 5C, with 1 μg/mL fMLP added, a current response of 27 nA appeared in the cell-in-lumen configuration, while no effective current change could be observed in the cell-out-electrode configuration. This also showed the advantage in the detection sensitivity of the PHF sensor towards low concentrations of releasing H_2O_2. Under the same drug additions, a bare electrode without cells was tested, no noticeable current changes were observed in Fig. 5D. In Fig. S4, the same volume of PBS without PMA or fMLP was also tested, no current change was noticed. Therefore, it can be inferred that the current signal generated in the presence of H1299 cells was attributed to H_2O_2 released from cells under the triggering of fMLP or PMA.

3.6. Detecting of H_2O_2 released from cells treated by DOX

Currently, PMA and fMLP are the most commonly used stimulating chemicals to trigger cell respiratory burst and rapid release of H_2O_2 for sensing. Compared with PMA or fMLP, anticancer drugs induced intracellular ROS generation is a cumulative process. It is more difficult to detect the trace amount of H_2O_2 released from cells in anticancer drug

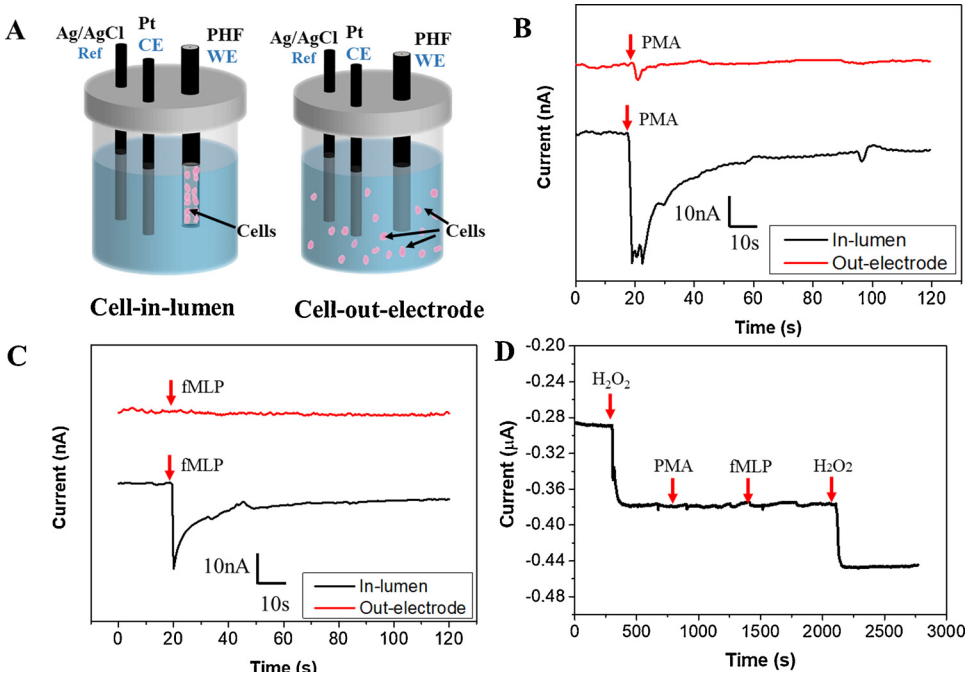

Fig. 5. (A) Schematic diagram of cell-in-lumen and cell-out-electrode experiment setups. (B) Amperometric responses of the PHF sensor to 0.14 μg/mL PMA in cell-in-lumen configuration and cell-out-electrode configuration. (C) Amperometric responses of the PHF sensor to 1 μg/mL fMLP in cell-in-lumen configuration and cell-out-electrode configuration. (D) Amperometric responses of the PHF sensor to successive addition of 1 μg/mL PMA, 1 μg/mL fMLP and 1 μM H_2O_2.

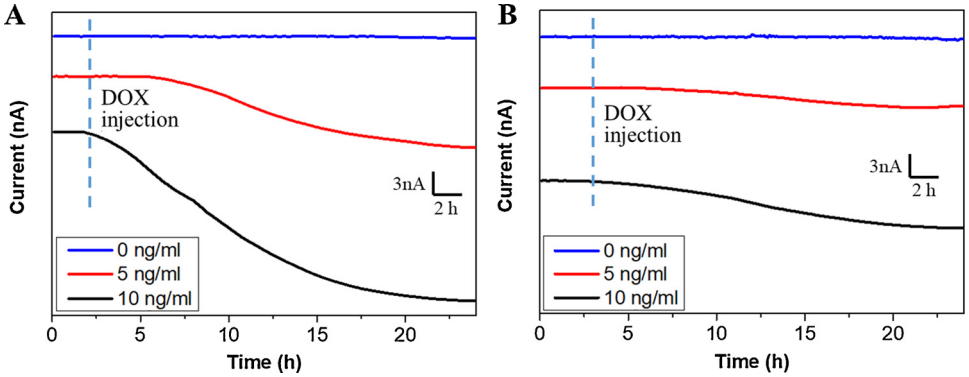

Fig. 6. (A) Amperometric responses of the PHF sensor in cell-in-lumen configuration. (B) Amperometric responses of the PHF sensor in cell-out-electrode configuration.

screening due to the spontaneous decomposition during the diffusion process. Doxorubicin (DOX) is a widely used chemotherapeutic agent, which can trigger cancer cell apoptosis with ROS generation [30,31]. Treating adherent cells with DOX causes a gradual H_2O_2 concentration elevation, but much slower than the respiratory burst induced by PMA or fMLP. Continuous monitoring of the entire H_2O_2 release process is essential for understanding the mechanism and toxicity of DOX in cancer therapies.

In this experiment, H_2O_2 continuous monitoring was performed by PHF cell-in-lumen and cell-out-electrode testing setups with the same number (1×10^5) of cells. Unlike the short-term respiratory burst as shown in Fig. 5, we used the PHF sensors for continuous H_2O_2 measurements for up to 24 h. In the cell-in-lumen configuration, the current in low dose group (5 ng/mL DOX) increased by 10 nA within 24 h, the current in high dose group (10 ng/mL DOX) increased by 22 nA, and the untreated group (0 ng/mL DOX) showed no apparent electrochemical current change. From the current curves in Fig. 6A, the H_2O_2 concentration change induced by DOX is a gradual accumulation process, not a burst release pattern as induced by PMA or fMLP. The microenvironmental H_2O_2 concentration of high DOX dose group reached 130 nM in 24 h. The H_2O_2 increasing trend matched with previous literature results by fluorescence analysis [32]. In cell-out-electrode configuration (Fig. 6B), the corresponding current increments of the three cases were 6 nA (10 nM DOX), 2 nA (5 nM DOX) and 0 nA (0 nM DOX) respectively. Based on the sensor's LOD, the cell-out-electrode configuration is incapable of being used in long-term drug screening. Table 1 listed analytical characteristics of our cell-in-lumen approach and other reported methods on detection of cellular H_2O_2. It could be found that our cell-in-lumen configuration greatly expanded monitoring duration while maintaining sensitivity. The explanation for the cell-in-lumen configuration in long-term monitoring that is superior to the conventional configuration can be attributed to the following two reasons: 1, The H_2O_2 released from cells diffuses or decomposed before reaching the sensor electrode. Shortening the transmission distance between the electrode and the cells can reduce the amount of H_2O_2 dissipation. 2, The suspended cells settle down within a few hours, causing a concentration gradient along the medium height. The PHF hollow structure fixed the spatial position of cells and electrode to minimize the influence of concentration gradients. These experiments further verified that such a cell-in-lumen PHF structure could stably detect the release of H_2O_2 from cells in long-term monitoring.

4. Conclusion

In summary, a hollow fiber sensor was developed to capture the released H_2O_2 in a flow-through mode, achieving highly sensitive detection of H_2O_2 at the nanomolar level. The cell-in-lumen configuration based on hollow fiber provided a stable sensing platform for real-time monitoring of H_2O_2 release. The HRP, AuNPs and MWNT were a conventional combination for H_2O_2 sensing, but the spatial structure redesign can significantly improve the performance of the traditional sensing materials. In short-term tests, the amplitude of the signal detected by the cell-in-lumen configuration was over six times as that of the cell-out-electrode method. More importantly, the high stability of PHF sensor made it possible to monitor the long-term process of drug-induced H_2O_2 release. This technology provided a general versatility for monitoring kinetic change of reactive small molecules and can be further applied in the fields of toxicity screening and drug development.

CRediT authorship contribution statement

Zhen Ma: Methodology, Investigation, Data curation, Writing - original draft. **Min Jiang:** Investigation. **Qin Zhu:** Investigation. **Ying Luo:** Investigation. **Gongxing Chen:** Investigation. **Min Pan:** Data curation. **Tian Xie:** Project administration, Resources. **Xiaojun Huang:** Validation, Writing - review & editing. **Dajing Chen:** Conceptualization, Project administration, Writing - review & editing, Resources.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Table 1
Comparison analytical performance of various cellular H_2O_2 sensors.

Sensor type	Linear range (μM)	Detection limit (nM)	Cell number	Monitoring duration	Spatial relationship	Ref.
Au-GO-HRP	0.02 – 1	7.5	2×10^5	200 s	In cell suspension	[20]
Pt-PEDOT	0.2 – 20	76	1×10^6	400 s	On electrode	[22]
Ag-PGN-HRP	0.00008 – 0.66	0.026	1×10^5	300 s	In cell suspension	[13]
MoS_2	0.1 – 100	2.5	2×10^6	250 s	In cell suspension	[17]
Pt-MoS_2	0.02 – 13	5	2×10^6	400 s	In cell suspension	[18]
Au-MWNT-PHF-HRP	0.01 – 5	6	1×10^5	24 h	In-lumen	This work

GO: Graphene oxide, PEDOT: poly(3,4-ethylenedioxythiophene), PGN: porous graphene.

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