Direct Electrodeposition of Bimetallic Nanostructures on Co-Based MOFs for Electrochemical Sensing of Hydrogen Peroxide

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Hydrogen peroxide (H₂O₂) is the most significant reactive oxygen species in biological systems. Here, we reported an electrochemical sensor for the detection of H₂O₂ on the basis of bimetallic gold-platinum nanoparticles (Au₃Pt₇ NPs) supported by Co-based metal organic frameworks (Co-MOFs). First, Au₃Pt₇ NPs, with optimal electrocatalytic activity and accessible active surface, can be deposited on the surface of the Co-MOF–modified glassy carbon electrodes (Au₃Pt₇/Co-MOFs/GCE) by one-step electrodeposition method. Then, the electrochemical results demonstrated that the two-dimensional (2D) Co-MOF nanosheets as the supporting material displayed better electrocatalytic properties than the 3D Co-MOF crystals for reduction of H₂O₂. The fabricated Au₃Pt₇/2D Co-MOF exhibited high electrocatalytic activity, and the catalytic current was linear with H₂O₂ concentration from 0.1 μM to 5 mM, and 5–60 mM with a low detection limit of 0.02 μM (S/N = 3). The remarkable electroanalytical performance of Au₃Pt₇/2D Co-MOF can be attributed to the synergistic effect of the high dispersion of the Au₃Pt₇ NPs with the marvelous electrochemical properties and the 2D Co-MOF with high-specific surface areas. Furthermore, this sensor has been utilized to detect H₂O₂ concentrations released from the human Hela cells. This work provides a new method for improving the performance of electrochemical sensors by choosing the proper support materials from diverse crystal morphology materials.

Keywords: electroanalysis, bimetallic nanoparticles, electrochemical sensor, hydrogen peroxide, cancer cells

INTRODUCTION

Hydrogen peroxide (H₂O₂), as one of the most significant reactive oxygen species (ROS), commonly exists in biological processes as a ubiquitous intracellular messenger or receptor signaling in various cells (Ushio-Fukai et al., 1999). On the one hand, H₂O₂ was considered as a toxic by-product during the process of aerobic metabolism (Rhee et al., 2005). On the other hand, some serious human diseases will be triggered by an abnormal concentration level of H₂O₂ (Trachootham et al., 2009; Yang et al., 2011), including myocardial infarct (Griending, 2004), Alzheimer’s disease (Markesbery, 1997), aging (Giorgio et al., 2007; Hayyan et al., 2016), and cancers (Jorgenson et al., 2013).
Therefore, developing an accurate and sensitive detection method to monitor the H$_2$O$_2$ concentration at the cellular level is vital and urgent for future clinic diagnosis and cancer treatment (Dong et al., 2019a). Until now, various analytical technologies have been utilized to detect H$_2$O$_2$, such as fluorescence (Ren et al., 2016; Ma et al., 2017), chemiluminescence (Yuan and Shiller, 1999; Xie and Huang, 2011), spectrophotometry (Yu et al., 2017), chromatography (Gimeno et al., 2015), and electrochemistry. Compared with the established analytical methods, the electrochemical sensing technique is a promising approach to attain dynamic analysis of H$_2$O$_2$ concentration for its outstanding properties, specifically high sensitivity, good selectivity, simple operation, and low cost.

Electrochemical sensors for testing H$_2$O$_2$ can be subdivided into natural enzymes and nanomaterial-based artificial enzymes (nanozymes)–based sensors. The natural enzymes were known as peroxidases, such as horseradish peroxidase (Sun et al., 2004). The enzyme-based electrochemical sensors have been widely utilized in electroanalysis for the advantage of specificity and fast response to the target substance. However, natural enzymes are restricted to their inherent weaknesses, including the uncontrollable deactivation and hardship in purification (Wang et al., 2018). Therefore, to overcome the shortages of natural enzymes, nanozymes have been taken into consideration as robust alternatives. Until now, a series of nanomaterials have been proved to possess intrinsic peroxidase-mimicking catalytic properties (Wu et al., 2019), including noble metals, metal oxides, and carbon-based nanomaterials (Wei and Wang, 2013).

Among various enzyme mimics, bimetallic nanoparticles (NPs) have been widely employed to fabricate electrochemical biosensors. It usually performed better electrocatalytic properties than its monometallic counterparts (Iyyamperumal et al., 2013; Li et al., 2020; Yang et al., 2021). As previously reported, the properties of electrons can be improved by the synergistic effects of each monometallic nanomaterials (Renner et al., 2006). However, few limitations are constraining the further application of these bimetallic particles–based electrochemical sensors: 1) it is usually a challenge to meet the satisfactory sensitivity of the living cells detection; and 2) their thermodynamic instability and tendency to aggregate, owing to their high surface free energy (Wang et al., 2019).

These disadvantages can be improved by immobilizing metal NPs in/on supporting structures. Recently, metal organic frameworks (MOFs) formed by the self-assembly of organic ligands and metal ions (Batten et al., 2013) have drawn extensive attention as supporting materials because of their inherent advantages (Furukawa et al., 2013). Among various MOFs, Co-based MOFs were widely used in fabricating electrochemical sensors as supporting material (Dong et al., 2019b). According to the previous research, the zeolite imidazolate framework (ZIF)–67 which compounded of the imidazole-based organic linkers and transition metal (Co) displaying a desirable 3D structure rhombic dodecahedron morphology, and it has been widely utilized for fabricating the 3D Co-MOF–modified electrodes. Lu et al. (2020) constructed a novel electrochemical sensor with the help of 3D Co-MOF to load artificial enzymes Ag nanostructures. Recently, two-dimensional (2D) Co-MOF has drawn extensive attention as supporting material in fabricating electrochemical sensors. The meso-tetra-(4-carboxyphenyl)–substituted porphyrins (TCPP) is the excellent ligand for the constructing 2D MOF due to their tetragonal symmetry and rigid structure (Zhao et al., 2018a). When the TCPP ligands connect with Co–2$, 2$D Co-MOF nanosheets was formed. Four Co paddle-wheel metal nodes link one TCPP ligand. These TCPP linked ultrasmall 2D metalloporphyrinic MOF nanosheets have been used to fabricating electrochemical sensors for electrochemical catalyzing and sensing applications (Zhao et al., 2015). For example, Huang et al. (2017) reported a novel sensor for catalyzing the cascade reactions. The electrochemical sensor was fabricated by peroxidase mimics material Au NPs and artificial glucose oxidase, ultrasmall 2D metalloporphyrinic MOF nanomaterial. Ma et al. (2019) reported a novel electrochemical sensor fabricated by TCPP-based 2D Cu-MOF nanosheets and Ag NPs. Because of the smaller size and more accessible active sites (Xu et al., 2016; Zhao et al., 2018b), 2D Co-MOF may combine other functionalized materials better than 3D bulk MOF crystals (Tan et al., 2017; Liu et al., 2020).

However, which morphology of MOF can perform better in loading artificial enzymes that still need more exploration?

In this work, our experiments first tried to answer whether AuPt NPs are a much better catalyst for H$_2$O$_2$ reduction reaction than either Au or Pt NPs. Then, we evaluated the electrocatalytic activity of 3D and 2D Co-MOF–modified electrodes, figuring out the influences of morphology for a supporting material. During the electrodeposition process with a negative voltage, AuPt NPs can be easily reduced from the solution adhering to the surface of the working electrode steadily (Wirtz and Martin, 2003). After comparing a series ration of Au NPs and Pt NPs with (V:V = 1:9, 3: 7, 5:5, 7:3, and 9:1), a novel sensor was constructed which bimetallic Au$_3$Pt$_7$ NPs decorated on the 2D Co-MOF–modified glassy carbon electrode (Au$_3$Pt$_7$/2D Co-MOFs/GCE) by electrodeposition. This high-performance electrochemical sensor was successfully utilized to monitor H$_2$O$_2$ concentration in real-time, and it performed a desirable property for tracing H$_2$O$_2$ in human cancer samples. Scheme 1 illustrated the detecting process of in suit analyzing of H$_2$O$_2$ secreted from Hela cells with drug stimulation by electrochemical sensor.

**EXPERIMENTAL**

**Reagents and Apparatus**

The reagents and apparatus were provided in detail in the Supporting Information.

**Synthesis of 2D and 3D Co-MOF**

In this work, two kinds of pristine Co-MOF materials were prepared according to the published method with some improvements (Zhao et al., 2015; Sun et al., 2020).

**Preparation of the Modified Electrodes**

First, 0.3 and 0.05 μM alumina slurries were used to polished the bare GCE (3 mm in diameter), until it shows mirror-like luster. Second, the electrodes were ultrasonicated in ultrapure water, ethanol, and ultrapure water. Last, nitrogen steam was used to dry the electrodes. A volume of 5 μl (2 mg/ml) of crystal ink of 2D Co-MOFs and 3D Co-MOFs were dropped on the GCE surface (the
modiﬁed electrode was labeled as 2D Co-MOFs/GCE and 3D Co-MOFs/GCE, respectively. Then, the electrodes were air-dried at room temperature. After that, HAuCl4 (1.0 mM) and H2PtCl4 (1.0 mM) were mixed with the different volume ratio. The Co-MOFs/GCE was immersed in the mixed HAuCl4 and H2PtCl4 solution. AuPt NPs were grown on the surface of the modiﬁed electrodes by electrodedepositing at −0.2 V (vs. Ag/AgCl). Last, two pristine Co-MOF-supported electrodes were fabricated, and these modiﬁed electrodes were washed by ultrapure water.

**Measuring H2O2 Secreted From Hela Cells**

In the electrochemical experiments at the cellular level, 10 mM phosphate-buffered saline (PBS) with N2-saturating (pH 7.0) was utilized as the electrolyte to collect the living Hela cells. The details of Hela cells culturing condition were listed in the Supporting Information. With the help of cyclic voltammogram (CV) measurement and amperometric I-t experiment, the redox characteristics of the fabricated electrodes Au3Pt7/2D Co-MOF/GCE were fully approved and the I-t experiment potential was kept at −0.5 V.

Before the analyzing experiments, the cultured cells were gently washed twice with PBS. Then, 3 ml of PBS was added into the Hela cells culture dish, and 400 μM ascorbic acid (AA) was injected into the dish to stimulate the cells releasing H2O2. With the amperometric technique, the current response for H2O2 detection was recorded.

**RESULTS AND DISCUSSION**

**Characterization of Noble Metal Nanostructures**

Here, we fabricated a bimetallic electrochemical sensor on the basis of Au3Pt7 NPs for H2O2 detection by CV measurements. Scanning electron microscopy (SEM) were employed to characterize the Au NPs, Pt NPs, and Au3Pt7 NPs. Figure 1A showed the SEM image of Au NPs that dispersedly immobilizing on the electrode. The Au NPs were of spheroidal nature, the average diameter of Au NPs was almost 20 nm. As recorded in the CV curves of Figure 1B, with different time of electrodeposition, the Au NP-modiﬁed electrodes showed an increased catalytic ability to H2O2, and the best catalytic response was obtained at 120 s. Figure 1C exhibited the histograms of the reduction peak current value of different electrodeposition time (t = 30, 60, 90, 120, and 150 s). The detection signal of H2O2 increased following the electrodeposition time, increasing until the electrochemical signal achieves a peak value of 120 s. Then, the current signal declined with continually increasing the electrodeposition time. The SEM image of Pt NPs is shown in Figure 1D, and the Pt NPs were of spheroidal nature, showing an average size of ~50 nm. Figures 1E,F demonstrate the CV curves with different electrodeposition time of Pt NPs. The peak current increased from 30 to 120 s and dropped down until 150 s. The result demonstrated that, with the electrodeposition time increased to 120 s, the detection signal of H2O2 reduction achieved a peak value. Therefore, 120 s was chosen to be the suitable electrodeposition time of synthesized Au3Pt7 NPs. As shown in Figure 1G, the SEM image of Au3Pt7 NPs was distributed sparsely on the surface of GCE electrode, where a uniform NP structure with nearly 100 nm exists. Figures 1H,I depict the volume ratio of AuPt NPs (V:V = 1:9, 3:7, 5:5, 7:3, and 9:1). Au3Pt7 NPs was selected as the optimum nanozyme for fabricating the novel electrochemical sensors. From the results above, metal NPs all gain high catalytic abilities to H2O2, especially the Au3Pt7 bimetallic NPs, and the phenomenon may come from the several reasons as follows: 1) electrodeposition provided a considerable route for its low cost,
immediate modification, stable current signal, and simple construction; and 2) Au3Pt7 NPs combining the advantages of the high catalytic ability of the Pt NPs and the stability of Au NPs. Therefore, the electrochemical catalytic capability and the selectivity of Au3Pt7 have been improved (Sun et al., 2017; Yu et al., 2018).

Characterization of Pristine 3D and 2D Co-MOF

The morphology analysis of 2D and 3D Co-MOF was investigated by SEM, transmission electron microscopy (TEM), powder X-ray diffraction (XRD), X-ray photoelectron spectroscopy (XPS), and Fourier transform infrared (FT-IR) spectroscopy. As Supplementary Figure S1 shown, the collected products of pristine Co-MOF exhibited a powder-like 3D Co-MOF that was purple and 2D Co-MOF that was brown, matching with the previous report. Figure 2A shows the SEM image of 3D Co-MOF, which displays a 3D structure rhombohedral dodecahedron crystalline morphology with the uniform size of nearly 500 nm. Figure 2B reveals the 2D Co-MOF with a sheet-like structure. The crumpled and wrinkled surface indicates its ultrathin property. The TEM image supported 2D Co-MOF with a thin nanosheet structure and confirmed the structure shown in the SEM image. Figures 2C,D reveal the XRD patterns of the 3D Co-MOF and 2D Co-MOF. Figure 2E shows the chemical composition and states of the Co-MOF. As indicated from the two lines of XPS data, there were two main peaks of approximately 780 and 796 eV for 3D and 2D Co-MOF products, respectively, corresponding to Co 2p3/2 and Co 2p1/2 that are derived from Co2+. The two broad peaks at 786.0 and 802.8 eV were satellite peaks, matching well with the reported data. Then, for further investigating of the prepared pristine MOFs, FT-IR spectroscopic studies are shown in Figure 2F, and the characteristic absorption peak of 3D Co-MOF and 2D Co-MOF nanosheet appeared at nearly 716, 770, and 1,025 cm⁻¹.
These three peaks can prove the existing of skeleton vibration absorption peak of the porphyrin ring (Bai et al., 2019). The peak approximately 1,396 cm$^{-1}$ is related to the stretching vibration of C=N. Besides, the peaks at 1,128 and 1,610 cm$^{-1}$ are generated by the vibration of the benzene ring skeleton outside the pyrrole ring. The absorption peak at 1,700 cm$^{-1}$ can be attributed to the C=O vibration of the carboxyl group on the benzene ring. These results demonstrated that the pristine Co-MOF products had been successfully synthesized.

The examination of CV and electrochemical impedance spectroscopy (EIS) characterized that the pristine Co-MOF of two morphologies was decorated on the surface of the surface of GCE. The electrochemical study of the fabricated sensor in 5 mM K$_3$Fe(CN)$_6$/K$_4$Fe(CN)$_6$ solution containing 0.1 M KCl (amplitude 10 mV, impedance spectral frequency 0.1~10$^5$ Hz). As shown in Figures 2G,H, the electrochemical performances of the interfacial of the modified electrodes have been tested by CV and EIS. For the bare GCE, a couple of reversible redox peaks was observed, and the current signals of 3D Co-MOF and 2D Co-MOF–modified electrodes (2D Co-MOF/GCE) were decreased. Moreover, the diffusion-limited process of the electrons was showed in the Nyquist plots. The semicircle diameter displaying the charge transfer resistance (Rct) of the modified GCE electrodes. The picture of EIS showed the bad conductivity of the pristine 3D Co-MOF. The Rct of 2D Co-MOF/GCE was close to 800 Ω, and the Rct of 3D Co-MOF/GCE was close to 1,700 Ω. Three-dimensional Co-MOF performed worse than the bare GCE in electrical conductivity properties. Without interlayer interactions and electron confinement, 2D Co-MOF performed better than 3D Co-MOF and bare GCE. As shown in Figure 2I by using the CV technique, the electrocatalytic activity of the selected two pristine Co-MOF for H$_2$O$_2$ reduction was evident, indicating the current signal of the 2D Co-MOF/GCE closed to 50 μA, the 3D Co-MOF/GCE closed to 23 μA, and the bare GCE was 8 μA. These results illustrated that Co-MOF materials had little influence in the
catalytic activity for H$_2$O$_2$ reduction in 100 mM PBS solution containing 10 mM H$_2$O$_2$, which can be ignored after the noble metal depositing on the Co-MOF-modified electrodes.

**Characterization of Au$_3$Pt$_7$ NP-Modified Co-MOF Electrodes**

After figuring out the optimum volume ratio of Au$_3$Pt$_7$/GCE for H$_2$O$_2$ detection, the influence of the supporting materials was investigated. Electrodepositing Au$_3$Pt$_7$ NPs on Co-MOF/GCE, the morphology of Au$_3$Pt$_7$/3D Co-MOF/GCE and Au$_3$Pt$_7$/2D Co-MOF/GCE was observed by SEM measurements. Moreover, the CV measurement results exhibited that 2D Co-MOF performed better in supporting that Au$_3$Pt$_7$ NPs showed the best electrocatalytic activity to reduce H$_2$O$_2$. As Figures 3A,B reveal, Au$_3$Pt$_7$ NPs immobilized on the 3D Co-MOF sparsely with an average size of 100 nm. The corresponding EDS spectrum verified the existence of Co, Au, and Pt elements, as shown in Figure 3C and Supplementary Figure S2. The pictures of SEM clarified that the Au$_3$Pt$_7$ NPs located on the 2D Co-MOF/GCE uniformly (Figures 3D,E). The result of EDS element analysis clearly characterized the Au$_3$Pt$_7$ NPs combining on the nanosheet 2D Co-MOF. The Co, Au, and Pt elements can be observed distinctly in Figure 3F and Supplementary Figure S3. The result supported that the Au$_3$Pt$_7$/2D Co-MOF/GCE was synthesized successfully. The small Au$_3$Pt$_7$ NPs attached better to the 2D Co-MOF nanosheet layers than these were attached to 3D Co-MOF. The redox peak current was increased due to the excellent conductivity of the Au$_3$Pt$_7$ NPs.

Operating in the solution of 5 mM K$_3$Fe(CN)$_6$/K$_4$Fe(CN)$_6$, in Figure 3G, after Au$_3$Pt$_7$ NPs depositing on two morphologies of pristine Co-MOF–modified electrodes, the gap between the cathodic and anodic peaks of the CV curves has been narrower, and the peak current of Au$_3$Pt$_7$ Co-MOF/GCE and Au$_3$Pt$_7$ 2D Co-MOF/GCE was increased. Comparing with the conventional 3D Co-MOF, the 2D Co-MOF nanosheets

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**FIGURE 3** | (A,B) SEM images of 3D Co-MOF. (C) EDS mapping analysis of Au$_3$Pt$_7$/3D Co-MOF/GCE. (D,E) SEM images of Au$_3$Pt$_7$/2D Co-MOF/GCE. (F) EDS mapping analysis of Au$_3$Pt$_7$/2D Co-MOF/GCE. (G) CV curves of GCE, Au$_3$Pt$_7$/3D Co-MOF/GCE, and Au$_3$Pt$_7$/2D Co-MOF/GCE in 5.0 mM K$_3$Fe(CN)$_6$/K$_4$Fe(CN)$_6$ solution containing 0.1 M KCl (amplitude 10 mV, impedance spectral frequency 0.1~10$^5$ Hz). (H) CV curves of GCE, 2D Co-MOF/GCE, Au$_3$Pt$_7$/3D Co-MOF/GCE, and Au$_3$Pt$_7$/2D Co-MOF/GCE in 100 mM PBS solution containing 10 mM H$_2$O$_2$ at a scan rate of 100 mV/s. (I) Histograms of GCE, 3D Co-MOF/GCE, 2D Co-MOF/GCE, Au$_3$Pt$_7$/GCE, Au$_3$Pt$_7$/3D Co-MOF/GCE, and Au$_3$Pt$_7$/2D Co-MOF/GCE in 0.1 M PBS containing 10 mM H$_2$O$_2$.
possessed a larger surface area, more accessible active sites for the substrate molecules (Li et al., 2018). As shown in Figure 3H, compared with the peak current of the pristine 2D Co-MOF-modified electrode, the current signal of the Au₃Pt₇/Co-MOF/GCE prominent increased, indicating that the H₂O₂ can be catalyzed effectively by the presence of Au₃Pt₇ NPs. Preliminarily, the 2D Co-MOF with the better affinity for locating the Au₃Pt₇ NPs was inferred. Two-dimensional MOF nanosheets possess unique advantages, such as their large surface area can facilitate the contact of substrate molecules with the active sites on their surface with minimal diffusion barriers (Huang et al., 2017), thus improving their performance in catalysis and sensing applications (Wang et al., 2016). Figure 3I illustrates the peak current of 10 mM H₂O₂ to electrodes modified by different materials. The sequence of the peak current increases as follows: GCE < 3D Co-MOF/GCE < 2D Co-MOF/GCE < Au₃Pt₇/3D Co-MOF/GCE < Au₃Pt₇/GCE < Au₃Pt₇/2D Co-MOF/GCE. The sequence indicated the 2D Co-MOF as supporting materials combining the Au₃Pt₇ NPs as the compound nanozymes can help get the best electrocatalytic activity to reduce H₂O₂.

These results are mainly attributed by the 2D Co-MOF with a highly porous structure and more accessible electroactive sites for adsorbing more Au₃Pt₇ NPs than the 3D Co-MOF. On the basis of the Randles–Sevcik equation, the electroactive surface area of different modified electrodes was calculated Ip = (2.69 × 10⁵) ADⁿ/²V¹/²C, where Ip is the peak current (A); A refers to the effective surface area of the electrode (cm²), D means the diffusion coefficient for [Fe (CN)₆]³⁻/⁴⁻ (6.7 × 10⁻⁶ cm² s⁻¹), n is the number of transition electrons of [Fe (CN)₆]³⁻/⁴⁻ (n = 1), γ means the scan rate (V/s), and C is the concentration of the redox reactant (5 × 10⁻⁶ mol cm⁻³). The effective surface area of Au₃Pt₇/2D Co-MOF/GCE was determined to be 0.143 cm², which is nearly 1.5 times higher than that of bare GCE (0.09 cm²). However, the effective surface area of Au₃Pt₇/3D Co-MOF/GCE was determined to be 0.085 cm², indicating the lousy conductivity of the 3D Co-MOF. Therefore, Au₃Pt₇/2D Co-MOF/GCE was selected to carry on the further electrocatalytic performance.

Electrochemical Performance of Au₃Pt₇/2D Co-MOF/GCE

The sensitivity of Au₃Pt₇/2D Co-MOF/GCE was verified in 10 ml of PBS solution with different concentrations of H₂O₂ (2, 4, 6, 8, and 10 mM) (Supplementary Figure S4A). The reduction peak current increased following the increase of H₂O₂ concentrations from 0 to 10 mM. The electrochemical signal was plotted, and a good linear relationship was revealed in Supplementary Figure S4B. These consequences preliminarily illuminated that the fabricated electrode possessed a good response for H₂O₂ reduction. Supplementary Figure S5A displays the effect of scan rates and the charge transport behavior of electrode Au₃Pt₇/2D Co-MOF/GCE that have been investigated, with the help of the [Fe (CN)₆]³⁻/⁴⁻ redox probe. The CV curves revealed that both the cathodic and anodic peak currents were enhanced with increasing scan rates from 50 to 700 mV/s. Furthermore, the current signal responses exhibited a proportional to the square root of the scan rates (Supplementary Figure S5B). The good linear relationship illustrated fast electron transfer kinetics with a typical diffusion-controlled process.

Amperometric Measurement of H₂O₂

For meeting the sensitivity of demonstrating H₂O₂ concentration at the cellular level, the suitable detecting potential of the fabricated electrode Au₃Pt₇/2D Co-MOFs/GCE needs to be obtained. The amperometric responses were recorded in different potentials from −0.2 to −0.6 V. With the potential increase, the current responses were enhanced under successive injection of 0.4 mM H₂O₂, and the solution was stirred continuously at 250 rpm. The highest response showed in the potentials −0.6 V, but the signal noise of the background currents was too larger to be ignored. On the basis of the above results, −0.5 V was selected as the optimal working potential in the following determination (Supplementary Figure S6).

The amperometric measurement was employed to evaluate the detection sensitivity of Au₃Pt₇/2D Co-MOFs/GCE for the reduction of H₂O₂. With the successive injection of different H₂O₂ concentrations into 10 ml of PBS at the optimum potential of −0.5 V. Figure 4A describes the current response for the low concentration of H₂O₂. A typical amperometric plot was exhibited of injecting H₂O₂ per 50 s. The insets of Figure 4A show that the current response was achieved at 0.1 μM. The result means that the electrochemical sensor had a good sensitivity for H₂O₂ at a low concentration. Figure 4B reveals the linear regions from 0.1 μM to 5 mM and the linear regression was I (μA) = −31.77C (mM) −0.16695 (R² = 0.999) with the sensitivities of 236.1 μA mM⁻¹ cm⁻². The amperometric plot of 5–60 mM was shown in Figures 4C,D, discovering the linear regression of high concentration of H₂O₂, which was I (μA) = −24.98C (mM) −73.26 (R² = 0.997) with the sensitivities of 174.6 μA mM⁻² cm⁻² (the sensitivities were calculated by dividing the slope of the linear regression equation by the geometric surface area of the bare GCE). In addition, the low detection limit (LOD) was calculated to be 0.02 μM (S/N = 3). The Au₃Pt₇/2D Co-MOF/GCE presented a good electrochemical catalytic activity for H₂O₂ with an extended linear range and a lower LOD.

Selectivity, Reproducibility, and Stability of Au₃Pt₇/2D Co-MOF/GCE

The selectivity of Au₃Pt₇/2D Co-MOF/GCE for H₂O₂ was evaluated by injecting the relevant species successively. As shown in Figures 4E,F, there was no obvious current signal when following adding different substances, including 5 mM L-AA, 5 mM DL-alanine, 5 mM glucose, 5 mM lactic acid, 5 mM citric acid, 5 mM Lysine acid, and 5 mM KCl, NaCl, and Na₂SO₄. However, an apparent electrochemical signal was observed when a low concentration of H₂O₂ (0.5 μM) was added into the same solvent system. These results indicated that the H₂O₂ sensor possessed high selectivity for H₂O₂.
The stability of the H$_2$O$_2$ sensor was investigated. The peak current of CV curves was displayed in Supplementary Figures S7A,B after 7 days, the signal of peak currents only decreased to 93.3%, which presented the excellent stability of the Au$_3$Pt$_7$/2D Co-MOF/GCE. The same sensor recorded five successive CV measurements. The response current signals shown in Supplementary Figure S8 were almost unchanged with a relative standard deviation of 3.5%, which suggested the excellent repeatability of the electrode. The relevant electrode materials for the electrochemical sensing of H$_2$O$_2$ displayed in Table 1. The novel electrode Au$_3$Pt$_7$/2D Co-MOF/GCE exhibited an acceptable electrochemical catalytic activity for H$_2$O$_2$ with an extended linear range and a lower LOD.

Real-Time Determination of H$_2$O$_2$ Released From Hela Cells

Hela cells have been elected as the model cell to estimate whether the sensitivity of the novel electro-sensor Au$_3$Pt$_7$/Co-TCPP/GCE could meet the need of evaluating H$_2$O$_2$ secreted from actual samples. Before the electrochemical detecting, dehydrogenate (DHE) staining was applied for preliminary analyzing the intracellular H$_2$O$_2$ of Hela cells. As the fluorescence image depicting, the intracellular concentration of H$_2$O$_2$ was kept at a low level before AA stimulation (Figure 5A). After the treatment with AA (400 μM), the level of endogenous H$_2$O$_2$ concentration increased apparently (Figure 5B). The DHE fluorescence photographs proved that H$_2$O$_2$ of Hela cells indeed motivated by AA.

The amperometric technique was utilized to analyze H$_2$O$_2$ at the cellular level with the potential of −0.5 V. AA was injected into the dish as a stimulant to boost the H$_2$O$_2$ secreted from the Hela cells. After the injection of 400 μM AA solution into the system without cell, there was a negligible current response. However, an obvious electrochemical current signal was observed when the same drug injected into the system.

**TABLE 1**| Comparison of the different H$_2$O$_2$ sensors in detection performance.

| Working electrode | Linear range (μM) | LOD (μM) | References |
|-------------------|-------------------|----------|------------|
| AgAuPt/ITO        | 4–4,000           | 2        | Peng et al. (2015) |
| Pt@Au/NWEs        | 0.15–3.2          | 0.12     | Liu et al. (2017)   |
| ZIF-67-Au@Pt/GCE  | 0.8–3.00          | 0.08     | Wang et al. (2021)   |
| MoS$_2$-Au@Pt/GCE | 10–19,070         | 0.39     | Zhou et al. (2017)  |
| PB/PtB@Pd/ITO     | 0.4–2,247         | 0.10     | Zhu et al. (2019)  |
| Pt@Au/EDA/GCE     | 1–450             | 0.18     | Li et al. (2013)  |
| Au$_3$Pt$_7$/2D Co-MOF/GCE | 0.1–5,000 | 0.02 | This work |
| ITO, indium tin oxide; NWEs, nanowire electrodes; EDA, ethylenediamine (C$_2$H$_8$N$_2$)
contained the Hela cells at the same concentration. The current value was calculated to be 204 nA, which corresponded to 0.12 \( \mu \)M \( \text{H}_2\text{O}_2 \) (Figure 5C). Bright images of Hela cells revealed that the modified electrodes and the electrochemical detection were harmless to the cells, and the morphology and viability of the living cells were similar to those without stimulating (Figure 5D). These results supported that \( \text{Au}_3\text{Pt}_7/2\text{D Co-MOF/GCE} \) could meet the need of the intracellular \( \text{H}_2\text{O}_2 \) in situ detection.

**CONCLUSION**

In conclusion, we have fabricated an enzyme-free electrochemical sensor for the real-time detection of \( \text{H}_2\text{O}_2 \) released from cells. We discovered that bimetallic \( \text{Au}_3\text{Pt}_7 \) NPs showed much higher electrocatalytic activity than pure \( \text{Au} \) NPs or \( \text{Pt} \) NPs toward \( \text{H}_2\text{O}_2 \) detection. Because of the ultrathin nanosheet structural features, 2D Co-MOF showed a better supporting ability than the 3D Co-MOF. Benefitting from the microplate structures of 2D Co-MOF and the high catalytic ability of \( \text{Au}_3\text{Pt}_7 \) NPs, we constructed a novel electrochemical biosensor on the basis of the \( \text{Au}_3\text{Pt}_7 \) NPs deposited on the 2D Co-MOF/GCE for the first time. The fabricated \( \text{Au}_3\text{Pt}_7/2\text{D Co-MOF/GCE} \) exhibited high electrocatalytic activity, fast response, and good sensitivity toward \( \text{H}_2\text{O}_2 \) reduction. The linear range of the \( \text{Au}_3\text{Pt}_7/2\text{D Co-MOFs/GCE} \) provided to \( \text{H}_2\text{O}_2 \) appealing in 0.1 \( \mu \)M–5 mM and 5–60 mM and exhibited a LOD of 0.02 \( \mu \)M (S/N = 3). These characters enable the real-time quantification of \( \text{H}_2\text{O}_2 \) secreted from Hela cancer cells under drug stimulation. This work provided a new method for improving the detection performance by changing the supporting materials of different crystalline morphologies. For future electrochemical sensors, more efforts will be made in cell adhesion/growth on the surface of the electrodes to get excellent sensitivity in situ detection of \( \text{H}_2\text{O}_2 \) or other disease biomarkers.

**DATA AVAILABILITY STATEMENT**

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding authors.

**AUTHOR CONTRIBUTIONS**

This study was conceived and supervised by DS, XL, and LZ. Synthesis, structural characterization, and electrochemical measurements were performed by YX, XS, and LC. The analysis of all data was done by YX and XS. The original draft was written by YX. The manuscript was revised by DS, JL, and LZ.
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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fchem.2022.856003/full#supplementary-material

Liu, C.-S., Li, J., and Pang, H. (2020). Metal-organic Framework-Based Materials as an Emerging Platform for Advanced Electrochemical Sensing. Coord. Chem. Rev. 410, 213222. doi:10.1016/j.ccr.2020.213222

Liu, Y., Zhang, Y., Hua, H., and Li, Y. (2017). Fabrication of Single Pt@Au Nanowire Electrodes for Monitoring Hydrogen Peroxide Released from Living Cells. RSC Adv. 7 (40), 44552–44558. doi:10.1039/c7ra08085a

Lu, J., Hu, Y., Wang, P., Liu, P., Chen, Z., and Sun, D. (2020). Electrochemical Biosensor Based on Gold Nanoflowers-Encapsulated Magnetic Metal-Org...
Ushio-Fukai, M., Alexander, R. W., Akers, M., Yin, Q., Fujio, Y., Walsh, K., et al. (1999). Reactive Oxygen Species Mediate the Activation of Akt/protein Kinase B by Angiotensin II in Vascular Smooth Muscle Cells. J. Biol. Chem. 274 (32), 22699–22704. doi:10.1074/jbc.274.32.22699

Wang, H., Chen, W., Chen, Q., Liu, N., Cheng, H., and Li, T. (2021). Metal-organic Framework (MOF)-Au@Pt Nanoflowers Composite Material for Electrochemical Sensing of H2O2 in Living Cells. J. Electroanalytical Chem. 897, 115603. doi:10.1016/j.jelechem.2021.115603

Wang, N., Sun, Q., and Yu, J. (2019). Ultrasmall Metal Nanoparticles Confined within Crystalline Nanoporous Materials: a Fascinating Class of Nanocatalysts. Adv. Mater. 31 (1), 1803966. doi:10.1002/adma.201803966

Wang, Q., Wei, H., Zhang, Z., Wang, E., and Dong, S. (2018). Nanozyme: an Emerging Alternative to Natural Enzyme for Biosensing and Immunoassay. Trac. Trends Anal. Chem. 105, 218–224. doi:10.1016/j.trac.2018.05.012

Wang, Y., Zhao, M., Ping, J., Chen, B., Cao, X., Huang, Y., et al. (2016). Bioinspired Design of Ultrathin 2D Bimetallic Metal-Organic-Framework Nanosheets Used as Biomimetic Enzymes. Adv. Mater. 28 (21), 4149–4155. doi:10.1002/adma.201600108

Wei, H., and Wang, E. (2013). Nanomaterials with Enzyme-like Characteristics (Nanozymes): Next-Generation Artificial Enzymes. Chem. Soc. Rev. 42 (14), 6060–6093. doi:10.1039/c3cs35486e

Wirtz, M., and Martin, C. R. (2003). Template-fabricated Gold Nanowires and Nanotubes. Adv. Mater. 15 (5), 455–458. doi:10.1002/adma.200390106

Wu, J., Wang, X., Wang, Q., Lou, Z., Li, S., Zhu, Y., et al. (2019). Nanomaterials with Enzyme-like Characteristics (Nanozymes): Next-Generation Artificial Enzymes (II). Chem. Soc. Rev. 48 (4), 1004–1076. doi:10.1039/c8cs00457a

Xie, J., and Huang, Y. (2011). Co3O4 Nanoparticles-Enhanced Luminol Chemiluminescence and its Application in H2O2 and Glucose Detection. Anal. Methods 3 (5), 1149–1155. doi:10.1039/c1ay05103b

Xu, R., Wang, Y., Duan, X., Lu, K., Micheroni, D., Hu, A., et al. (2016). Nanoscale Metal-Organic Frameworks for Ratiometric Oxygen Sensing in Live Cells. J. Am. Chem. Soc. 138 (7), 2158–2161. doi:10.1021/jacs.5b13458

Yang, J.-C., Lu, M.-C., Lee, C.-L., Chen, G.-Y., Lin, Y.-Y., Chang, F.-R., et al. (2011). Selective Targeting of Breast Cancer Cells through ROS-Mediated Mechanisms Potentiates the Lethality of Paclitaxel by a Novel Diterpene, Gelomulide K. Free Radic. Biol. Med. 51 (3), 641–657. doi:10.1016/j.freeradbiomed.2011.05.012

Yang, S., Han, G., Chen, Q., Yu, L., Wang, P., Zhang, Q., et al. (2021). Au-Pt Nanoparticle Formulation as a Radiosensitizer for Radiotherapy with Dual Effects. Ijn Vol. 16, 239–248. doi:10.2147/ijn.S287523

Yu, M., Zhao, K., Zhu, X., Tang, S., Nie, Z., Huang, Y., et al. (2017). Development of Near-Infrared Ratiometric Fluorescent Probe Based on Cationic Conjugated Polymer and CdTe/CdSe QDs for Label-free Determination of Glucose in Human Body Fluids. Biosens. Bioelectron. 95, 41–47. doi:10.1016/j.bios.2017.03.065

Yu, Y., Yu, C., Niu, Y., Chen, J., Zhao, Y., Zhang, Y., et al. (2018). Target Triggered Cleavage Effect of DNAzyme: Relying on Pd-Pt Alloys Functionalized Fe-MOFs for Amplified Detection of Pb2+. Biosens. Bioelectron. 101, 297–303. doi:10.1016/j.bios.2017.10.006

Yuan, J., and Shiller, A. M. (1999). Determination of Subnanomolar Levels of Hydrogen Peroxide in Seawater by Reagent-Injection Chemiluminescence Detection. Anal. Chem. 71 (10), 1975–1980. doi:10.1021/ac981357c