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Multiple self-cleaning paper-based electrochemical ratiometric biosensor based on the inner reference probe and exonuclease III-assisted signal amplification strategy

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1 **Multiple self-cleaning paper-based electrochemical ratiometric**
2 **biosensor based on the inner reference probe and exonuclease**
3 **III-assisted signal amplification strategy**

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Abstract

Rapid and accurate detection of nucleic acids plays a major role in biological research and clinical diagnostics. Here, a 3D multiple self-cleaning electrochemical ratiometric microfluidic paper-based analytical device (SER- μ PAD) has been constructed for manganese super oxide dismutase (MnSOD) gene detection on the basis of the inner reference probe and exonuclease III (Exo III)-assisted analytes recycling amplification method. To simplify manual operations, a multipath self-cleaning tab that could manipulate fluid transport was introduced into the paper-based device, realizing time-programmable self-cleaning of the electrode. For achieving sensitive detection of MnSOD gene, the methylene blue (MB)-modified capture probe (CP) as the inner reference element was first self-assembled on triangular Au nanosheets modified paper working electrode to provide a built-in correction and improve the detection accuracy. When MnSOD gene existed, it hybridized with the hairpin-structured signal probe, triggering the cyclic amplification with the assistance of Exo III selective digestion to engender numerous residual DNA labeled with ferrocene (Fc) that could be captured on electrode surface by CPs. Hence, the Fc tags were close to the electrode surface, resulting in the oxidation peak current of Fc (I_{Fc}) increase, while that of MB (I_{MB}) was constant on account of the unchanged distance between the MB tags and the electrode. The value of I_{Fc}/I_{MB} was linear with MnSOD gene concentration from 10 nM to 1200 nM, and the detection limit was 3.91 nM. This strategy provides an accurate, robust, and sensitive method for nucleic acids detection and shows great potential in the construction of portable devices.

Keywords: electrochemical, ratiometric, paper-based analytical device, manganese super oxide dismutase gene, inner reference element

1. Introduction

Nucleic acids play an important role in the expression of genetic information in all organisms (Xiong et al., 2017). However, even very small changes in sequences of nucleic acids can have great influence in biomedical and biological domains, such as population genetics and human diseases (Xiong et al., 2017; Zheng et al., 2018a). As a result, sensitive, rapid, and selective detection of nucleic acids is fundamental important in food monitoring, clinical diagnosis, and genetic analysis (Hu et al., 2019; Niemz et al., 2011; Yang et al., 2018). Of late, a variety of sensor techniques, including electrochemistry (Deng et al., 2017; Sun et al., 2018), electrochemiluminescence (Chai et al., 2010; Liu et al., 2017), colorimetry (Baeissa et al., 2010; Deng et al., 2008), and photoelectrochemistry (Zang et al., 2014; Saha et al., 2018) have been proposed for nucleic acids detection. Among them, the biosensors based on the electrochemical techniques are particularly attractive owing to their merits of low cost, relatively low power consumption, quick responses, and amenability to miniaturization (Blair et al., 2019; Cho et al., 2019; Hu et al., 2018; Zheng et al., 2018b).

However, a primary obstacle to hinder the broader practical application of electrochemical biosensors is the problem associated with the accuracy, stability, and reproducibility caused by the inevitable variations in electrode surface. This may result in the initial background signals on the electrode differing from each other. Although the relative signal changes induced by the targets can be detected for each electrode (Bonham et al., 2012), it is time-consuming and inconvenient. In addition, whether the measured signal changes arise from the targets binding or deterioration of electrode surface is uncertain. In response, ratiometric sensors with inner reference element, which possess the ability to provide a built-in correction, have been studied to enhance the performance of the sensors. (Zhuang et al., 2014; Zhuang et al., 2016). Deng et al. proposed an electrochemical biosensor based on the ratiometric strategy and the dual signal-tagged probe for tumor marker detection (Deng et al., 2017). Du et al. demonstrated a robustness ratiometric biosensor based on the combination of two electrochemical probes (Du et al., 2014). Although these approaches can efficiently

improve the accuracy, stability, and reproducibility of the biosensors, the sensitivity is not satisfactory because of the limitation of conventional 1:1 binding ratio of aptamers to targets. Thus, exploring an efficient signal amplification strategy that can circumvent the above limitation and make full use of the superiority of inner reference probe plays an important role in the construction of a sensitive, user-friendly, and portable point-of-care (POC) device.

As one of the promising platforms to construct POC devices, microfluidic paper-based analytical device (μ PAD) has attracted widespread attention since its first proposal by Whitesides group (Martinez et al., 2007). Considering the intrinsic merits of paper, such as excellent biocompatibility, ease of operation, inexpensive, and ease of production, μ PAD holds the great potential to launch a revolution in environmental monitoring, food hygiene safety, and disease diagnostics, especially in the areas where the resource is limited (Li et al., 2019; Li et al., 2018; Tian et al., 2017; Wang et al., 2018). Unfortunately, two major drawbacks affecting potential development of μ PAD are poor electrical conductivity and cumbersome manual washing steps. The *in-situ* growth of noble metal nanomaterials on cellulose fibers is a widely used method to improve the conductivity of chromatography paper (Zhang et al., 2018). In the meantime nanomaterials with relatively large surface area also play a crucial role in enhancing electro-active surface area of paper. So in this work, the homogeneous triangular Au nanosheets (T-AuNSs) were grown on naked paper *in situ* through a simple chemical reaction, effectively ameliorating the performance of paper. To overcome the limitation of manual washing of the working zone and be convenient for non-specialists to use, a new-type multipath self-cleaning tab was designed by modulating geometries of the pre-set path as well as capillary action and laminar flow on paper, achieving the time-programmable cleaning of the paper working electrode (PWE).

In this work, a simple self-cleaning μ PAD with inner reference probe was constructed for manganese super oxide dismutase (MnSOD) gene detection by superimposing exonuclease III (Exo III)-assisted analytes cyclic amplification in electrochemical dual-signals ratiometric strategy. As shown in Scheme 1, the

methylene blue (MB)-modified hairpin capture probe (CP) as the inner reference element was self-assembled onto T-AuNSs modified PWE (T-AuNSs/PWE) via Au-S bonds. The T-AuNSs not only had excellent conductivity to boost signal responses, but also had large electro-active surface area to immobilize more biological ligands, widening the linear range. In the presence of targets, the hairpin conformation of signal probe (SP) labeled with ferrocene (Fc) would switch from close to open state, reforming the double strands with a blunt 3' terminus. Then, Exo III could identify this 3' terminus and initiate enzymatic cleavage of SP to generate residual DNA with Fc accompanied by the releasing of MnSOD gene to take part in the next cycle. Subsequently, the residual DNA was captured on PWE surface by CPs, resulting in an increase in the oxidation peak current of Fc (I_{Fc}), while the peak current of MB (I_{MB}) was almost constant due to the unchanged distance between the MB tags and PWE. In the electrochemical sensing system, the detection sensitivity of the targets has been promoted efficiently by means of introducing Exo III-assisted cyclic amplification strategy and using T-AuNSs/PWE as an excellent sensor platform. Moreover, the self-cleaning process notably simplified the manual steps and reduced operation errors. In view of the aforementioned distinguishing features, the 3D self-cleaning electrochemical ratiometric μ PAD (SER- μ PAD) fabricated in this study is superior to the conventional ratiometric biosensor, and it has great potential application in POC testing.

Scheme 1

2. Experimental section

2.1. Design of the SER- μ PAD

The shape of SER- μ PAD was drawn by Adobe Illustrator CS4 and then the designed patterns were printed on Whatman No.2 chromatography paper by wax printing techniques (Giokas et al., 2014; Jin et al., 2015; Zhang et al., 2016). The definition of colors in the CMYK code was shown in Table S1. Next, the patterned paper was baked in an oven at 135 °C for about 2 min to make sure that the wax could permeate through the paper to create hydrophobic barriers shown in Fig. S1 and S2. As illustrated in Fig. S3, the 3D SER- μ PAD consisted of a detection tab (30 mm $\times$ 30

mm), an auxiliary tab (30 mm × 30 mm), a multipath self-cleaning tab (30 mm × 45 mm), and an adsorptive tab (45 mm × 30 mm). The detection tab had a bulb-shaped hydrophilic area. After T-AuNSs were modified on this area, a second layer of wax (8.0 mm × 2.0 mm) was printed on the PWE, followed by melting the wax using the above method to generate a controllable circular zone (diameter of 10 mm) for layer-by-layer modification (Scheme 1A, part a). The carbon counter electrode and the Ag/AgCl reference electrode were screen-printed on auxiliary tab. The multipath self-cleaning tab contained one semi-hydrophilic circular zone (zone I), one hollow circular zone (zone II) with the diameter of 10 mm, and three special semi-hydrophilic paths numbered 1-3, respectively. The semi-hydrophilic path 3 had a new open channel crafted with a blade longitudinally to control the time of fluidic transport. And the procedures were elaborated in Supplementary Material. Prior to self-cleaning process, the detection tab needs to be folded into the middle of the multipath self-cleaning tab and an adsorptive tab as shown in Scheme 1A, part b. And the picture of the real product was added in Fig. S4A. As shown in Fig. S5, after washing buffer was added into zone I, it would arrive at zone II at different times along the three paths, respectively. Owing to the precise alignment of the zone II and the working electrode, the buffer solution would flow to the PWE by means of gravity actions. Then the solution diffused on the whole PWE to wash off the uncombined DNAs. Under the suction of hydrophilic paper fiber, the effluent flowed to the following adsorptive tab, completing multiple self-cleaning processes. At last, as illustrated in Scheme 1A, part c and Fig. S4B, after folding along the line between the detection tab and auxiliary tab, a complete electrochemical circuit was formed once phosphate-buffered solution (PBS) was added.

2.2. Preparation of the T-AuNSs/PWE

The detailed preparation procedures of T-AuNSs/PWE were described in Supplementary Material.

2.3. Fabrication and assay procedures of the μ PAD

Prior to assembling on T-AuNSs/PWE surface, 50 μ L of 50 μ M CP was dissolved in 950 μ L of 34 mM Tris-HCl including 8.5 mM KCl, 1.7 mM CaCl_2 , 233

mM NaCl, 1.7 mM MgCl₂, and 10 mM tris(2-carboxyethyl)phosphine (pH 7.4), and then the mixed solution was incubated under darkness at room temperature for 60 min to cleave S-S bonds. Subsequently, 50 μ L of freshly prepared CP was added on the T-AuNSs/PWE, followed by covering the treated PWE with an opaque plastic box to slow the evaporation process. Then, the assembly was incubated at 25 °C overnight (15 h). Afterward, in order to block the nonspecific sites as well as to get the well-aligned DNA monolayer, 50 μ L of 1 mM 6-mercaptohexanol (MCH) was dropped into the surface of CP/T-AuNSs/PWE for 1 h at ambient. The resulting sensing platform was marked as MCH/CP/T-AuNSs/PWE. In parallel, the Exo III-assisted signal amplification was performed in centrifuge tubes, using 66 mM Tris-HCl buffer solution (0.66 mM MgCl₂, pH 8.0) for this enzyme. Briefly, 5 μ M SP, various specified concentrations of target solution, and Exo III (150 U) were mixed together and incubated at 37 °C for 2 h. Afterward, the solution was heated up to 75 °C for 600 s to inactivate the Exo III. After the MCH/CP/T-AuNSs/PWE was immersed in 50 μ L of the reaction solution for 2 h, the μ PAD was folded into the configuration shown in Scheme 1A, part b. PBS (10 mM, pH 7.4) was added into zone I to achieve self-cleaning process, and the electrode was dried under nitrogen atmosphere. The electrochemical capability of the modified electrodes was investigated with square wave voltammetry (SWV) (potential ranges, -0.4 to 0.4 V; step potential, 4 mV; frequency, 5 Hz; amplitude, 25 mV) in 10 mM PBS (pH 7.4). Meanwhile, the control assays for single-base mismatched DNA (Sm-DNA) or scramble DNA (Sc-DNA) were also performed at exactly the same experimental conditions. All SWV measurements were carried out at room temperature and at least three times.

3. Results and discussion

3.1. Characterization of the T-AuNSs/PWE

The morphologies of chromatography paper and Au nanomaterials were characterized by scanning electron microscope (SEM). The naked chromatography paper has unique 3D hierarchical framework that enables the substrate to accommodate a variety of active materials (Fig. 1A and 1B). To construct a desirable

electrochemical platform, T-AuNSs were grown on cellulose fibers. As indicated in Fig. 1C and D, numerous AuNSs are uniformly grown on cellulose fibers due to the strong interaction between the celluloses and AuNSs based on the *in-situ* growth mechanism. And the morphology of Au nanomaterial can be directly observed in enlarged SEM image (Fig. 1E). The AuNSs are triangular and have large surface area, which provides lots of active sites for biological ligands immobilization. The energy dispersive X-ray (EDX) elemental mapping analysis reveals the distribution of the elements of Au, C, and O, which is further confirmed by the energy dispersive spectrometer (EDS) spectrum (Fig. 1F). Besides, the successful preparation of T-AuNSs can also be demonstrated via X-ray diffraction (XRD). As depicted in Fig. 1G, four representative diffraction peaks at 38.2, 44.4, 64.6, and 77.5 are observed in the XRD pattern of T-AuNSs/PWE (red curve) comparing with that of the bare paper (blue curve). They can be indexed to the (111), (200), (220), and (311) crystallographic planes of Au cube structure respectively (JCPDS card no.04-0784). All the results provide a solid evidence for the successful growth of T-AuNSs on cellulose fibers. To investigate the conductivity of the designed T-AuNSs/PWE, a four-probe tester is used to measure its sheet resistance shown in Fig. 1H. Then, the resistivity ρ is calculated as $6.3 \times 10^{-6} \Omega \text{ cm}$ according to the formula $R_s = \rho/d$, where R_s is the sheet resistance and d is the thickness of T-AuNSs. This result indicates that the prepared T-AuNSs/PWE has excellent conductivity and T-AuNSs can accelerate the electron transfer efficiently to improve the sensitivity. The successful *in-situ* growth of T-AuNSs has greatly ameliorated the performance of paper, which paves the way for the development of flexible electronics.

Fig. 1

3.2. Electro-active area evaluation of the T-AuNSs/PWE

After growing noble metal nanomaterials *in situ* on cellulose fibers, the resulting substrate not only reserves the original flexibility of the cellulose matrix, but also has large electro-active area (Sun et al., 2018; Zhang et al., 2018). In order to evaluate the electro-active surface area of T-AuNSs/PWE, the cyclic voltammetry measurements were carried out in 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ solution. As shown in Fig. 2A, the presence

of a pair of well-shaped redox peaks is consistent with a reversible electrochemical behavior (Cinti et al., 2018). And a slight increase in the peak-to-peak separation is observed, indicating rapid electrons transfer occurred at the surface of T-AuNSs/PWE. In addition, good linear correlations between the peak currents and the square root of scan rates are obtained (Fig. 2B), suggesting that the mass transfer process in this system is controlled by diffusion. As a consequence, according to the slope of I versus $v^{1/2}$, the electro-active surface area of T-AuNSs/PWE can be calculated as 0.853 cm^2 using the Randles-Sevcik equation, which is 1.85 times larger than that of the Au nanoparticles modified PWE (0.461 cm^2) (Fig. S6). The method of calculating the electro-active surface area was described in Supplementary Material. These results indicate that the T-AuNSs effectively enhanced the electro-active surface area of the PWE and the T-AuNSs/PWE can be an excellent substrate for the fabrication of an electrochemical biosensor.

Fig. 2

3.3. Feasibility validation

The conformations of the SP and CP oligonucleotides were analyzed using Mfold web server (the detail was depicted in Supplementary Material). To further testify the feasibility of SER- μ PAD, a series of stepwise modified PWEs were characterized by SWV method. As displayed in Fig. 3A, after immobilization of CP and MCH, there is a distinct oxidation peak of MB at -0.252 V on account of the effective electron transfer of MB on T-AuNSs/PWE (curve a). Next, with the introduction of residual DNA, a remarkable Fc response current at approximately 0.196 V appears, whereas MB response current is kept constant. This may be presumably due to the unchanged distance between the MB tag and the surface of T-AuNSs/PWE (curve b), indicating that the MB label can provide a built-in correction to improve the detection accuracy. Thus, the target concentration can be monitored on the basis of the ratio responses. Based on the above results, it clearly demonstrates that the μ PAD has been manufactured successfully and can be used to detect MnSOD gene.

3.4. Characterization of the electrochemical μ PAD

Electrochemical impedance spectroscopy (EIS) is an effective tool to characterize the stepwise fabrication processes of the PWEs (Bardea et al., 1999; Deng et al., 2009; Zhang et al., 2019b). In a typical spectrum, the semicircular diameter is associated with the resistance of the interfacial charge-transfer (R_{ct}) (Cao et al., 2012; Zhang et al., 2019a). As is illustrated in Fig. 3B, the paper modified by T-AuNSs has a relative small R_{ct} value (18.25 Ω , curve a), which is attributed to the super conductivity of AuNSs and the large electro-active area of T-AuNSs/PWE. However, the value of R_{ct} is increased to 240.2 Ω (curve b) after assembling CP on T-AuNSs/PWE due to the repulsive interaction between the negatively charged CP and $[\text{Fe}(\text{CN})_6]^{3-/4-}$. Next, passivation of the electrode surface with MCH leads to a significant enhancement of R_{ct} value (438.6 Ω , curve c). After incubating with residual DNA solution, the R_{ct} value increases sharply to about 963.2 Ω (curve d), indicating the residual DNA is immobilized on the PWE through hybridization process.

In a cyclic voltammogram (CV), the peak currents as well as the separation of peak potentials can also be used to characterize the different modified electrodes. As can be seen in Fig. 3C, a pair of well-defined and enhanced redox peaks of $[\text{Fe}(\text{CN})_6]^{3-/4-}$ (Liu et al., 2018) can be clearly observed for T-AuNSs/PWE (curve a), indicating that T-AuNSs can accelerate electron transfer efficiently. Then, the self-assembly of CP onto PWE leads to an obvious decrease in the peak current (curve b) due to the presence of CP blocking the diffusion of $[\text{Fe}(\text{CN})_6]^{3-/4-}$ towards the electrode surface. When the remaining active sites on CP/T-AuNSs/PWE are blocked by MCH, the peak currents further decrease and the separation of peak potential becomes larger than before (curve c). At last, after introducing the residual DNA into the sensing platform, a smaller peak current is obtained and the peak-to-peak separation continuously increases because of the hybridization between the residual DNA and CP. These results of CVs are in conformity with the EIS measurements, indicating the electrochemical sensor has been successfully fabricated based on Scheme 1B.

Fig. 3

3.5. Electrochemical detection of MnSOD gene

The MnSOD gene encodes the specific enzyme for regulating the reactive oxygen species generated by the cell metabolism (Sarsour et al., 2014; Dhar et al., 2012). And it was chosen as the target to verify the practicability of the μ PAD. Under optimal conditions, a satisfactory sensitivity was obtained since the Exo III-assisted cleavage allowed the target DNA to recycle many times, producing lots of residual DNA for ratiometric detection in PBS. As shown in Fig. 4A, the peak current of Fc increases gradually with the increasing MnSOD gene concentration whereas the responses of MB are almost unchanged to promise the accuracy, reproducibility, and stability of different electrodes. Fig. 4B displays a well-behaved linear response between the value of I_{Fc}/I_{MB} and MnSOD gene concentration in the range from 10 nM to 1200 nM ($I_{Fc}/I_{MB} = 0.217 + 3.90 \times 10^{-4} c$, $R = 0.997$) with a calculated detection limit of 3.91 nM ($S/N = 3$) (López-Marzo et al., 2018). In comparison with the previously reported strategies for nucleic acids detection (Lin et al., 2019; Wang et al., 2017), the proposed electrochemical strategy reveals a wider linear range. What's more, the SER- μ PAD developed in this work is simpler and more portable than other biosensors (Li et al., 2016; Wu et al., 2013), which provides a new avenue for the development of easy-to-use POC devices that meet the ASSURED (Affordable, Sensitive, Specific, User-friendly, Robust and rapid, Equipment-free, Deliverable) criteria.

Fig. 4

3.6. Reproducibility, stability, and specificity of the SER- μ PAD

The reproducibility of the SER- μ PAD was evaluated to make sure its practical application. Five PWEs with the same modification conditions were selected for the detection of MnSOD gene, and the relative standard deviation was 3.81% (Fig. 5A). Besides, after the biosensor was stored at 4 °C for 15 days, as much as 90.5% of the initial response to the same target concentration was remained (Fig. 5B). These indicate that the reproducibility and stability of the biosensor was acceptable.

In addition, additional two kinds of DNA sequences, including Sm-DNA and Sc-DNA, were selected to investigate the specificity of the μ PAD. As displayed in Fig.

5C and 5D, the I_{FC}/I_{MB} value is significantly obvious in the presence of 700 nM MnSOD gene. Nevertheless, the signal caused by the Sc-DNA (700 nM) is similar to the signal response of blank solution, which can be attributed to the fact that the Sc-DNA cannot hybridize with SP. Sm-DNA (700 nM) generates much lower electrochemical signal compares with the MnSOD gene on account of the lower hybridization efficiency between Sm-DNA and SP. These findings demonstrate that the SER- μ PAD has a satisfactory selectivity for nucleic acids detection.

Fig. 5

3.7. Application of the μ PAD in real samples

To evaluate the applicability and reliability of the proposed μ PAD, the recovery tests were carried out by adding a series of different concentrations of MnSOD gene into the 10-fold diluted healthy human serum samples (obtained from Shandong Tumor Hospital, China). As shown in Table S2, the obtained recoveries for the added MnSOD gene are 95.8%, 92.7%, and 93.5%, respectively. It demonstrates that possible interferences from the serum are negligible. These results indicate that the SER- μ PAD has great promising application for MnSOD gene detection in real biological samples.

4. Conclusions

In summary, a novel 3D electrochemical μ PAD for MnSOD gene detection has been successfully constructed. A unique breakthrough of this work is the ingenious design of a multipath self-cleaning tab, achieving the time-programmable cleaning of the working zone. Besides, an efficient integration of Exo III-assisted targets recycling method and a dual-signaling ratiometric strategy with an inner reference element greatly improved the sensitivity, reproducibility, and stability of the biosensor. This paper-based device can be easily applied for the arbitrary targets' detection just by simply changing the corresponding DNA sequences, which is expected to be more widely used in clinical diagnostics and personalized healthcare, especially in resource-limited regions.

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Figure caption

Scheme 1. (A) Assembly and operation illustration of the SER- μ PAD. (B) Schematic diagram of the SER- μ PAD for target detection.

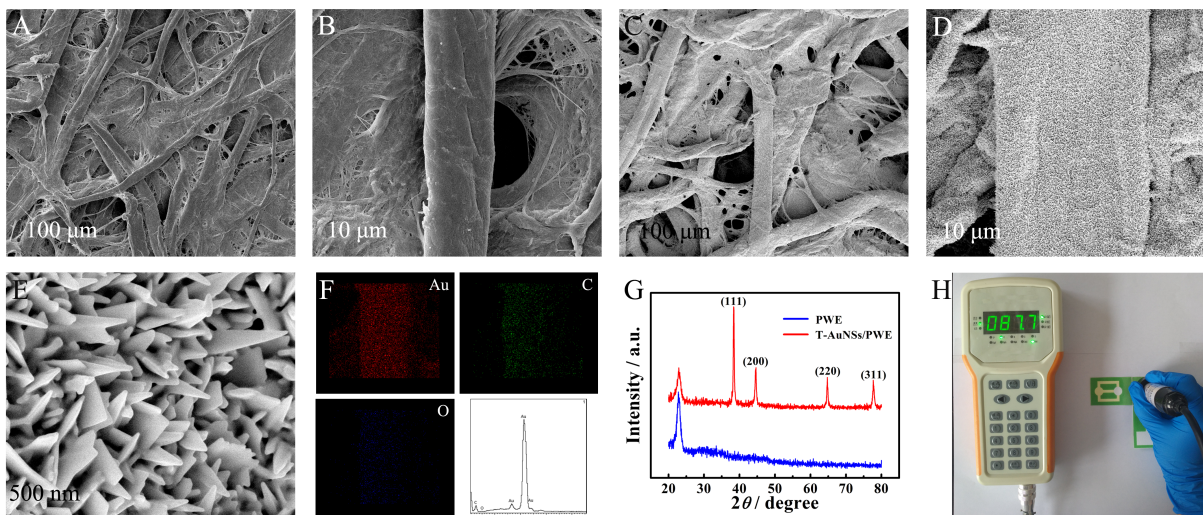
Fig. 1. SEM images of (A, B) the naked paper, and (C-E) T-AuNSs/PWE at different magnifications. (F) The element mapping and EDS image of T-AuNSs/PWE. (G) XRD patterns of naked paper (blue curve) and T-AuNSs/PWE (red curve). (H) The sheet resistance of T-AuNSs/PWE.

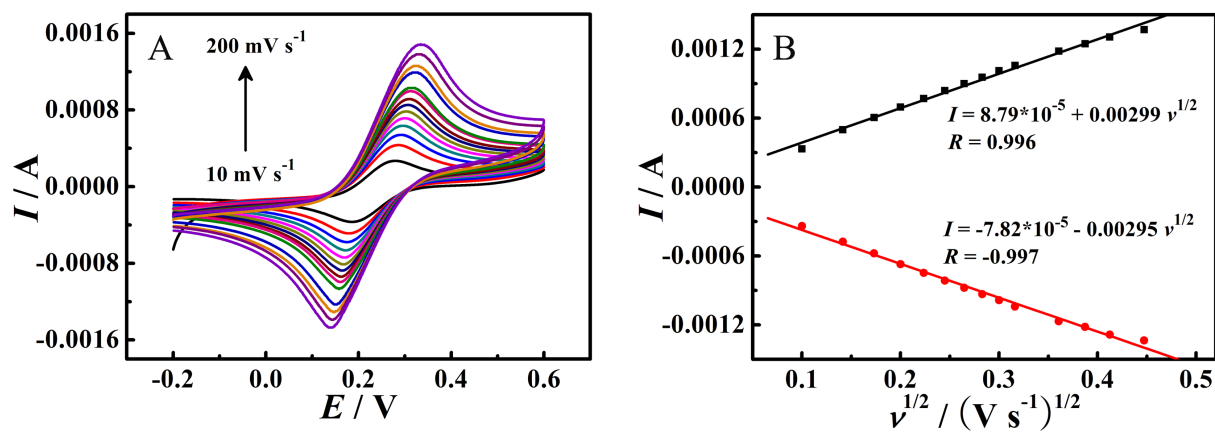
Fig. 2. (A) CVs of T-AuNSs/PWE in 5.0 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ containing 0.1 M KCl at various scan rates (10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 130, 150, 170, 200 mV s^{-1}). (B) Plots of the oxidation and reduction peak currents vs. square root of scan rates.

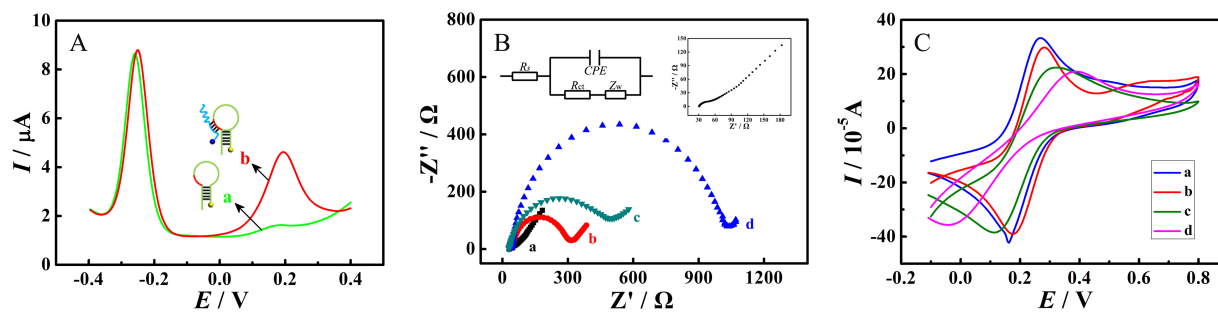
Fig. 3. (A) Feasibility of the μ PAD. SWV responses of the MCH/CP/T-AuNSs/PWE before (curve a) and after being treated with the reaction solution of 500 nM MnSOD gene (curve b). EIS (B) and CVs (C) of the stepwise modified PWEs in 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ aqueous solution containing 0.1 M KCl: (a) T-AuNSs/PWE, (b) CP/T-AuNSs/PWE, (c) MCH/CP/T-AuNSs/PWE, and (d) residual DNA/MCH/CP/T-AuNSs/PWE.

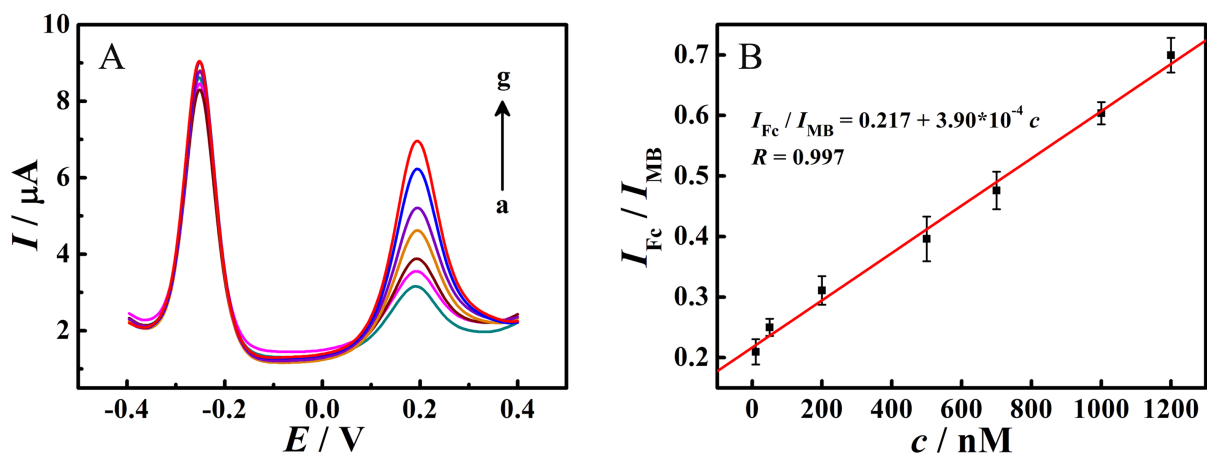
Fig. 4. (A) SWV responses of the biosensor to different concentrations of MnSOD gene (a-g: 10, 50, 200, 500, 700, 1000, 1200 nM). (B) The calibration curve of $I_{\text{Fc}}/I_{\text{MB}}$ versus the MnSOD gene concentration from 10 to 1200 nM. Error bars represent the standard deviations of three independent experiments.

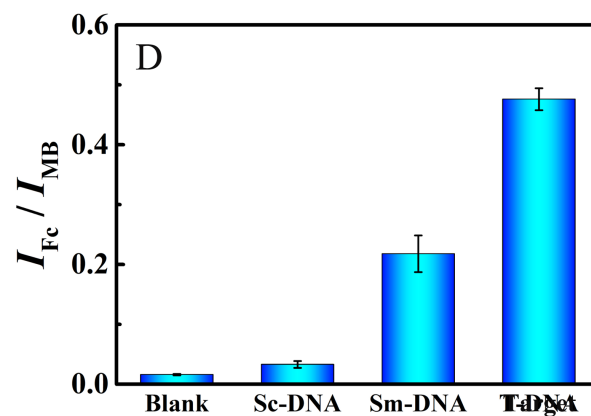
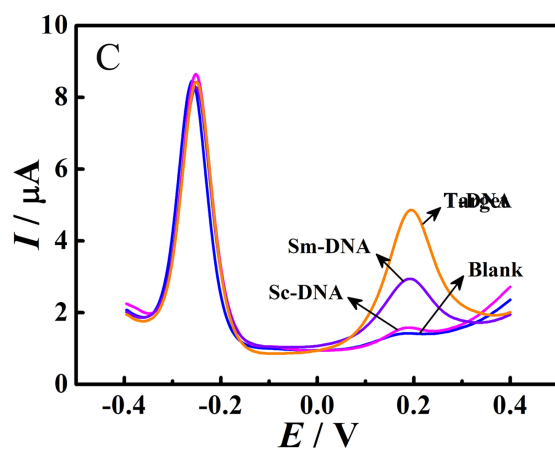
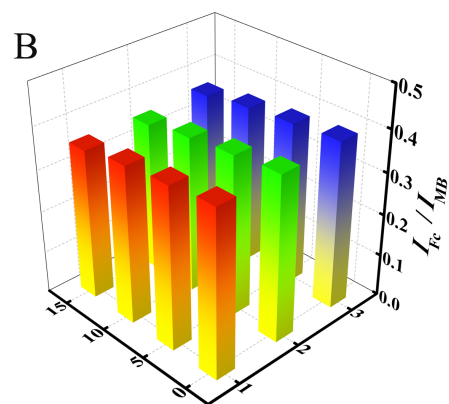
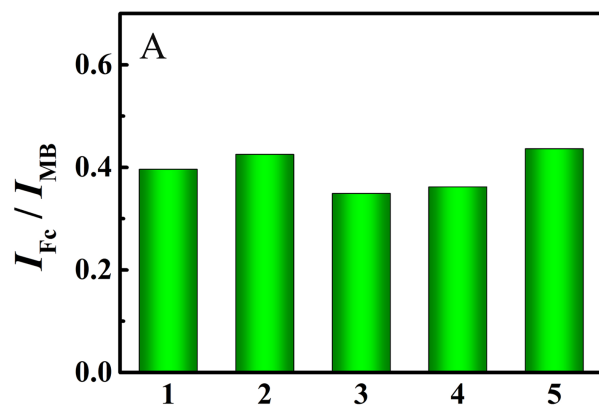
Fig. 5. (A) The reproducibility of the proposed SER- μ PAD to 500 nM MnSOD gene. (B) The stability of μ PADs in the presence of MnSOD gene (500 nM). (C) SWV curves of the MCH/CP/T-AuNSs/PWE in PBS without, and with Sc-DNA, Sm-DNA, and MnSOD gene. (D) The values of $I_{\text{Fc}}/I_{\text{MB}}$ for the blank solution (10 mM PBS) and various DNA sequences.

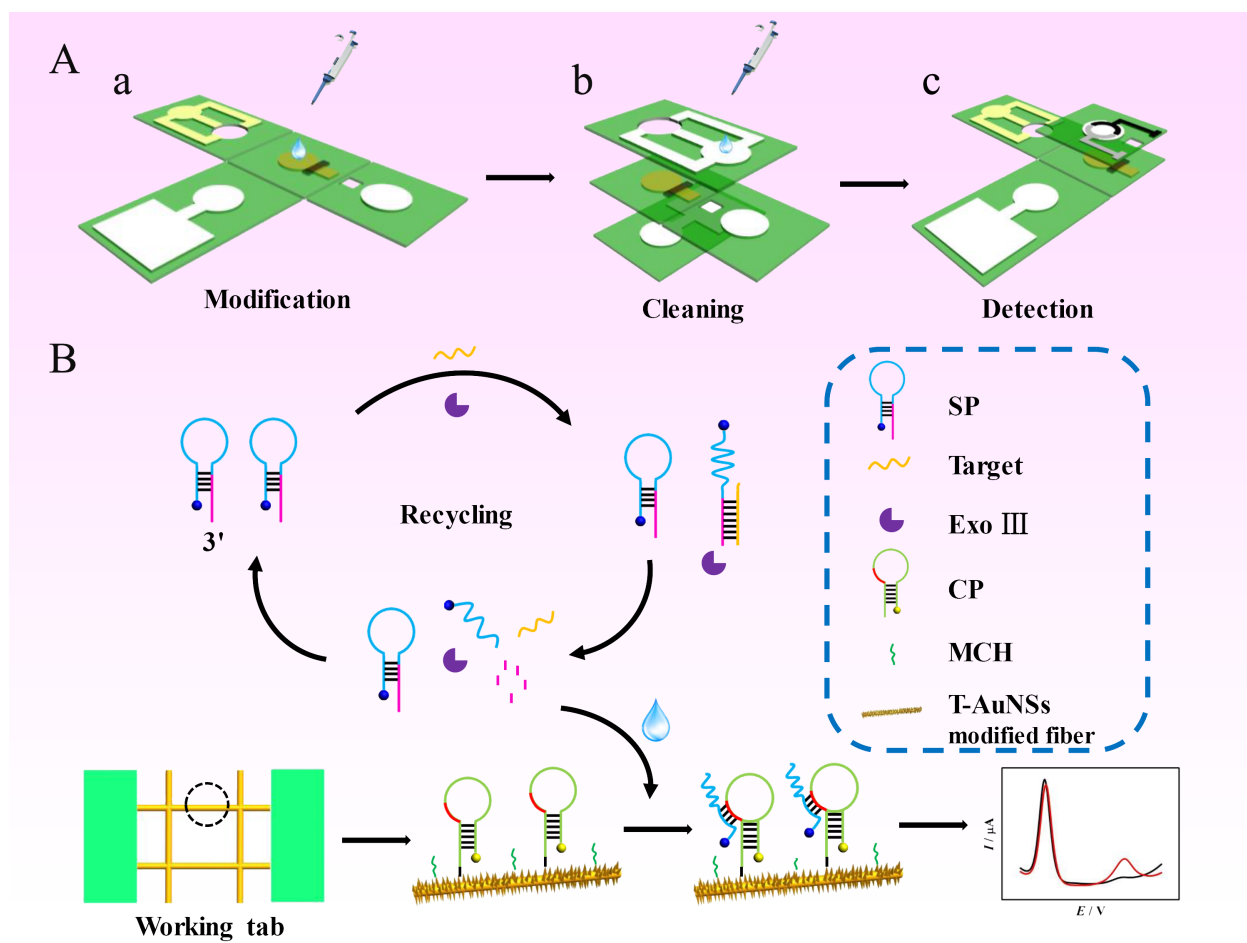












Highlights

- 1) The T-AuNSs were successfully grown in situ on the cellulose fibers.
- 2) A multipath self-cleaning tab was designed to achieve self-cleaning process.
- 3) An inner reference probe was introduced to improve the reproducibility.
- 4) Exo III-aided analytes cyclic amplification greatly improved the sensitivity.
- 5) The device showed great potential in the construction of portable devices.

The authors declare that they have no competing interests.

Journal Pre-proof

Credit author statement

Xiaoxiao Zheng: Conceptualization, Methodology, Investigation, Writing-Original Draft. **Li Li:** Investigation, Writing-Reviewing and Editing, Resources. **Lina Zhang:** Formal Analysis, Visualization, Data Curation. **Li Xie:** Resources, Data Curation. **Xianrang Song:** Resources, Visualization. **Jinghua Yu:** Funding Acquisition, Project Administration.

Declaration of interests

☒ 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.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: