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Sensitively electrochemical detection of the DNA damage *in situ* by electro-Fenton reaction based on Fe@Fe_2O_3 core-shell nanonecklace and multi-walled carbon nanotube composite

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ABSTRACT

An electrochemical biosensor for mimicking the metal-mediated DNA damage pathway *in situ* was presented. The Fenton reagents (H_2O_2 and iron ion) for the DNA damage were generated *in situ* with a constant rate from the sensing film. H_2O_2 and iron ion reacted further together to yield hydroxyl radical, which attacked DNA in the film. These courses of DNA damage were just like those happened in organism. The DNA damage was detected by monitoring the differential pulse voltammetric response of an electrochemical indicator, $Co(phen)_3^{3+}$. Another electrochemical indicator, $Ru(NH_3)_6^{3+}$, was also used for monitoring the DNA damage as a complementary means and the minimal detectable amount of DNA damage was 0.16 μg. The biosensor had good reproducibility. After this biosensor was used for 11 times, 90% of the first detection signal was obtained.

1. Introduction

Some transition metals such as Cr(VI), Ni(II), Be, Cd(II), As(III) compounds, Co(II) and Fe(II)-nitrilotriacetate have been confirmed to be human carcinogens or possibly carcinogenic according to the International Agency for Research on Cancer [1]. A number of studies have shown that the transition metal ions cause DNA damage through the production of reactive oxygen species (ROS) (frequently via Fenton-type reactions) under aerobic conditions.

Methods for detecting DNA damage could serve as effective screening for the toxicity of the chemicals at an early point in their commercial development and as an effective means for checking genetic disease. The DNA damage products have been identified and quantified by many methods and techniques [2], such as gas chromatography/mass spectrometry, high-performance liquid chromatography, ^{32}P-post labeling, gel electrophoresis, and immunoassay. Although these assays are powerful for the detection of DNA damage, the drawbacks are also obvious, such as low throughput, long duration, and high demand for manpower. Recently, the electrochemical and electrochemiluminescent sensors and sensor arrays for the rapid detection of DNA damage have also been aroused more and more attention [3–7].

Electrochemical methods offer a rapid and relatively inexpensive approach to detect DNA damage and hybridization. In electrochemical methods, the classic Fenton reaction was usually used because it could generate the extremely reactive hydroxyl radical (•OH), a representative of ROS, which could induce several classes of DNA damage, including single-strand break, double-strand break, abasic sites, and base oxidation [8]. Guo et al. incorporated glucose oxidase into a multi-component photoelectrochemical sensor film and generated H_2O_2 *in situ* in this film in the presence of glucose. When the test sample contained Fe^{2+}, Fe^{2+} reacted with H_2O_2 and generated hydroxyl radicals by the Fenton reaction [7].

The classic Fenton reactions are usually used in treating the organic pollutants of the wastewater. However, the classic Fenton reaction has some intrinsic disadvantages, such as narrow pH range (pH 3.0–4.0) and high cost of H_2O_2, during dealing with the pollutants of the wastewater. These drawbacks were also existed when it was used in the detection of DNA damage. In order to overcome these drawbacks, the electro-Fenton (E-Fenton) process on the basis of the combined use of iron ions and cathodically generated hydrogen peroxide has been developed [9–12]. For example, using Fe@Fe_2O_3 core-shell nanowires and multi-walled carbon nanotubes (MCNTs) as a novel E-Fenton system, Ai et al.

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studied the degradation of rhodamine B during wastewater treatment [4]. In our previous study, the E-Fenton system of $Fe@Fe_2O_3$ core-shell nanonecklaces and Au nanoparticles has been used to study the DNA damage [13]. However, we found that the sensitivity of this sensor was not high enough. Therefore, it is still necessary to develop high sensitive and stable biosensor for DNA damage.

In this paper, a new DNA damage electrochemical biosensor was developed by incorporating the mixture of MCNTs and $Fe@Fe_2O_3$ nanonecklace into a membrane containing poly(dimethyldiallylammonium chloride) (PDDA) and intact DNA. In the membrane, the DNA damage was caused by the hydroxyl radicals. Under slightly acidic conditions, the hydroxyl radicals were produced during the reaction of iron ions leaking from $Fe@Fe_2O_3$ nanonecklace and H_2O_2 cathodically generated on MCNTs. Both the generation of hydroxyl radicals and the whole course of DNA damage were completed in the membrane, which were just like the course of DNA damage caused by heavy metals in organism. Using $Co(phen)_3^{3+}$ and $Ru(NH_3)_6^{3+}$ as electrochemical indicators, the DNA damage course was monitored by the measurement of the differential pulse voltammetric (DPV) signal change. This biosensor has good stability and high sensitivity. It may be a good tool for studying the mechanisms of DNA damage pathways *in vivo*. MCNTs used here are more stable than the gold nanoparticle used in our previous work [13]. The latter is more suitable for studies on the DNA damage induced by photo-Fenton reaction owing to its absorption to ultraviolet light. Moreover, this biosensor is more sensitive than the biosensor using the gold nanoparticle for the DNA damage detection.

2. Experimental

2.1. Materials

Herring sperm DNA (ds-DNA) and PDDA were purchased from Sigma. $Ru(NH_3)_6Cl_3$ was purchased from Aldrich. MCNTs were purchased from Shenzhen Nanotech Port Co., Ltd. and dispersed in ultrapure water. $Co(phen)_3(ClO_4)_3{\cdot}3H_2O$ was synthesized according to the published procedures [14]. $Fe@Fe_2O_3$ core-shell nanonecklace was synthesized as follows [15]: 0.15 g of $FeCl_3{\cdot}6H_2O$ was dissolved in 50 mL of ultrapure water to form a ferric solution (solution 1) and 0.3 g of $NaBH_4$ was added to 20 mL of ultrapure water to form solution 2. Solution 2 was then dropped into the solution 1 at the rate of 0.5 mL s^{-1}. During the addition, the fluffy black precipitates appeared on the surface of the solution. The fluffy black precipitates were washed with ultrapure water and ethanol, and finally dried under nitrogen ambience. Phosphate buffer solution (PBS) was prepared by mixing suitable amount of 0.1 M NaH_2PO_4 and 0.1 M Na_2HPO_4 and the different pH values were adjusted using 0.1 M H_3PO_4 or 0.1 M NaOH. All reagents are of analytical grade and used without further purification. Ultrapure water was used in all experiments.

2.2. Instrumentation

The electrochemical experiments were carried out using a CHI 660 C electrochemical workstation (Shanghai CH Instruments, China) with a three-electrode arrangement consisting of a glassy carbon electrode (GCE) or film-coated GCE working electrode, a saturated calomel reference electrode (SCE) and a platinum wire auxiliary electrode. Scanning electron microscopy (SEM) images and transmission electron microscopy (TEM) images were performed on a JEM-2000EM scanning electron microscope (JEOL, Japan) and a JSM-6700 transmission electron microscope (JEOL, Japan), respectively. The UV–vis absorption spectra were obtained on a Cary 50 probe spectrophotometer (Varian, Australia). The DNA damage by cathodic treatment processes was performed using a potentiostat/galvanostat (Shenzhen Zhaoxin Electronic Equipments & Instruments Producer, China). The pH values of all solutions were measured with a pHS-25C acidometer (Shanghai Leici Instrument Factory, China). Ultrapure water was prepared with an Aquapro AWL-1002-P (Ever Young Enterprises Development Co., Ltd, China).

2.3. Procedures

2.3.1. Film construction

After the GCE had been pretreated as previously reported [16], the sensor film was constructed by sequentially covering GCE with PDDA, the mixture of $Fe@Fe_2O_3$ core-shell nanonecklaces and MCNTs with a volume ratio of 10:30, PDDA and ds-DNA. The concentrations of them in sequence were 2.0 mg mL^{-1}, 1.5 g L^{-1}, 2.0 mg mL^{-1} and 2.0 mg mL^{-1}, respectively, and the used amount of each layer was 8.0 μL. The electrode was gently washed with water and air-dried at room temperature after casting a layer. Because the layer of PDDA was positively charged and the ds-DNA was negatively, the ds-DNA could be immobilized on the film electrostatically. The final film was denoted as DNA/PDDA/$Fe@Fe_2O_3$–MCNTs/PDDA. Scheme 1 shows the composition of the multi-membrane detection method of DNA damage induced by the E-Fenton reaction. During the course of the cathodic treatment, the oxygen in the solution permeated the multi-membrane and was reduced to H_2O_2 on the surface of MCNTs. Then H_2O_2 reacted with iron ion, which was generated by slowly leaking from $Fe@Fe_2O_3$ core-shell nanonecklace, to produce $^{\bullet}OH$. The $^{\bullet}OH$ attacked the DNA in the film and induced DNA damage *in situ*. The DPV peak current difference between before (Scheme 1a) and after (Scheme 1b) the DNA damage with $Co(phen)_3^{3+}$ as the probe was used as the measurement signal for the DNA damage.

2.3.2. DNA damage by E-Fenton reaction and electrochemical measurements

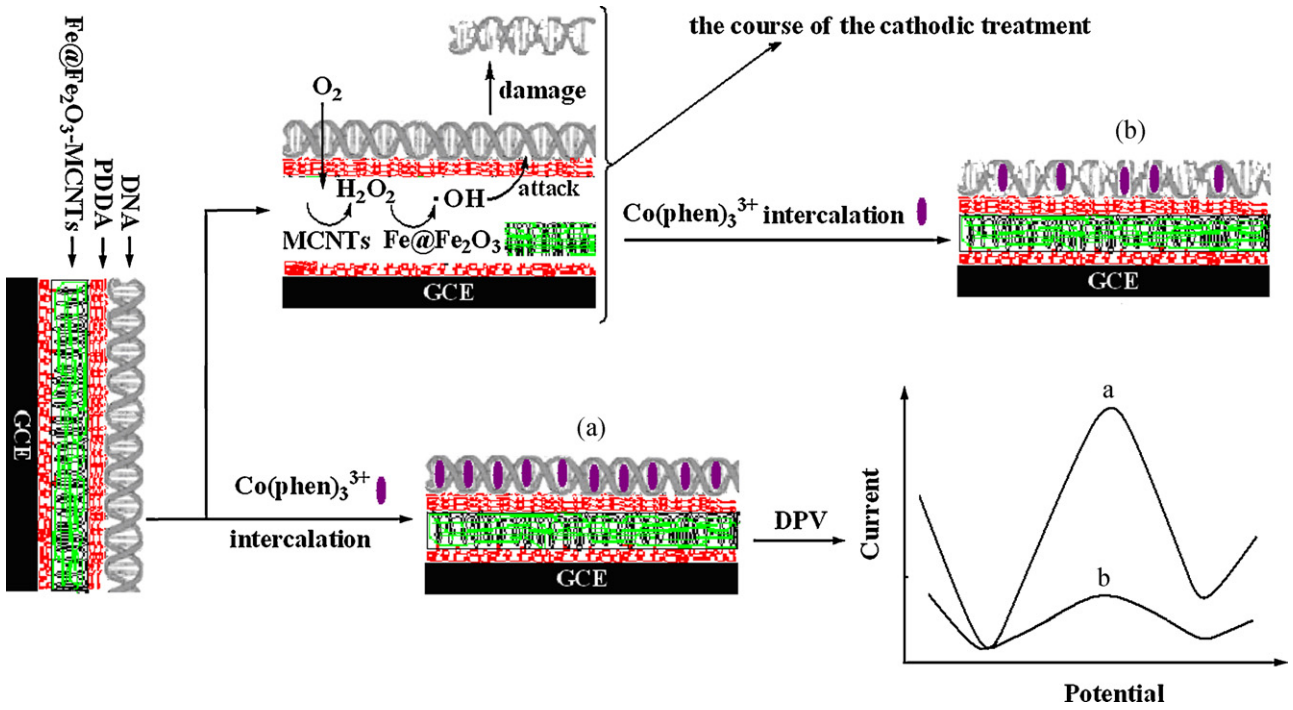
The DNA damage by E-Fenton processes was performed in 0.1 M of pH 5.5 PBS using a potentiostat. During the experiments, the cathode was fed with fresh air. The potential between the anode and the cathode was controlled at 1.2 V. Pt wire was used as the anode with the diameter of 0.05 mm. The film-coated electrode was used as the working cathode. After a given time, the electrode was taken out and rinsed with water. Then the electrode was placed into a cell containing 20 mM $Co(phen)_3^{3+}$ and 0.1 M PBS of pH 5.5. The DPV of $Co(phen)_3^{3+}$ was obtained and used as signal of the DNA damage. The conditions of DPV were as follows: pulse amplitude 10 mV; pulse width 100 ms, and pulse period 300 ms. The scan potential range was from 0.6 V to −0.4 V. The solution was purged with nitrogen and a nitrogen atmosphere was maintained for voltammetry. $Ru(NH_3)_6^{3+}$, another electrochemical probe, was used to verify the conclusion obtained from $Co(phen)_3^{3+}$ and the scan potential range was from 1.2 V to 0.4 V, and other parameters were the same as used in $Co(phen)_3^{3+}$ experiments.

3. Results and discussion

3.1. Characterization of $Fe@Fe_2O_3$ core-shell nanonecklace and film-coated electrode

3.1.1. The micro-structure of the $Fe@Fe_2O_3$ core-shell nanonecklace on the surface of the electrode

The micro-structure of $Fe@Fe_2O_3$ core-shell nanonecklace was investigated by SEM (Fig. 1a) and TEM (Fig. 1b and c). It was found that the diameters of the nanonecklaces were in the



Scheme 1. Schematic diagram illustrating the DNA damage induced by the E-Fenton reaction and the detection method.

range of 50–150 nm from the SEM. They looked like earthworms and entwined together forming many pores. Such loose structure had a large specific surface area and strong adsorbility, which made electrolyte and gas be adsorbed and pass through the pores very easy. The TEM images showed that they looked like a peal necklace which was formed by the connection of nanospheres. The diameters of nanosphere were 50–150 nm. The nanostructures had gray edge and dark center, which suggested that they be core-shell structure. The results are consistent with those of the reference and the XRD spectrum confirmed that the prepared nanomaterials were constituent with Fe and Fe_2O_3 [15].

3.1.2. Monitoring the fabrication processes of the film

By studying the cyclic voltammetry of 1.0 mM $[Fe(CN)_6]^{3-/4-}$ (1:1) containing 0.1 M KCl on the electrode, the fabrication processes of the film were monitored and the results are shown in Fig. 2. At the bare GCE (curve a), $[Fe(CN)_6]^{3-/4-}$ (1:1) had a well-defined cyclic voltammogram with the cathodic peak current i_{pc} of 1.985×10^{-5} A and the anodic peak current i_{pa} of -1.941×10^{-5} A, and the difference (ΔE_p) between the cathodic peak potential (E_{pc}) and the anodic peak potential (E_{pa}) was 104 mV. After a layer of PDDA had been immobilized on the surface of GCE, both i_{pc} and i_{pa} of $[Fe(CN)_6]^{3-/4-}$ increased (curve b) due to the affinity of the positively charged PDDA to $[Fe(CN)_6]^{3-/4-}$. Because $Fe@Fe_2O_3$ core-shell nanonecklace and MCNTs had good conductivity and large surface area, the peak currents increased further (curve c) with the assembly of the mixture of nanomaterials on the surface of GCE/PDDA. After another layer of PDDA had been modified, a pair of much larger peaks (i_{pc} 6.778×10^{-5} A and i_{pa} -4.495×10^{-5} A) and a smaller ΔE_p (74 mV) were obtained (curve d). After ds-DNA had been modified on the electrode, the i_{pc} and i_{pa} became 4.253×10^{-5} A and -2.191×10^{-5} A, respectively, and the ΔE_p became larger (134 mV) (curve e) because the repulsion between DNA and $[Fe(CN)_6]^{3-/4-}$ was existed.

3.2. Electrochemical measurements of the DNA damage

The DNA damage *in situ* induced by the E-Fenton reaction could be detected by DPV with 20 mM $Co(phen)_3^{3+}$. Owing to the intercalative binding of $Co(phen)_3^{3+}$ to ds-DNA, $Co(phen)_3^{3+}$ bound more strongly to intact ds-DNA than the damaged DNA. After the sensor had been treated by E-Fenton reaction for a given time, the DPV of $Co(phen)_3^{3+}$ on the biosensor was obtained and the results are shown in Fig. 3A. When the biosensor had not been treated by the E-Fenton reaction (Fig. 3A, 0 min), the largest peak at 0.19 V was obtained owing to the largest amount of $Co(phen)_3^{3+}$ bound to the DNA. Then the peak current decreased with the prolongation of the E-Fenton reaction time, which suggested that the ability of the film to bind the probe be gradually losing (Eqs. (1)–(4)). After the biosensor had been treated by the E-Fenton reaction for 30 min, a new small peak appeared at −0.05 V due to the reduction of iron ions leaching from $Fe@Fe_2O_3$ core-shell nanonecklace.

$$\text{ds-DNA} + Co(phen)_3^{3+} \xrightarrow{\text{intercalate}} \text{ds-DNA–}Co(phen)_3^{3+} \text{ complex} \quad (1)$$

$$\text{ds-DNA–}Co(phen)_3^{3+} + e^- \rightarrow \text{ds-DNA–}Co(phen)_3^{2+} \text{ at electrode} \quad (2)$$

$$\text{ds-DNA} \xrightarrow{\bullet OH} \text{DNA(damaged)} \quad (3)$$

$$\text{DNA(damaged)} + Co(phen)_3^{3+} \not\longrightarrow \text{intercalate difficultly} \quad (4)$$

The DNA damage could also be detected using another electrochemical indicator, $Ru(NH_3)_6^{3+}$. After the biosensor had been treated by the E-Fenton reaction, an increasing DPV peak current was obtained with the prolongation of the E-Fenton reaction time (see Fig. 3B). $Ru(NH_3)_6^{3+}$ could catalyze the oxidation of guanines. After the ds-DNA in this film had been damaged, guanine was exposed from the protection of the double helix, which made it

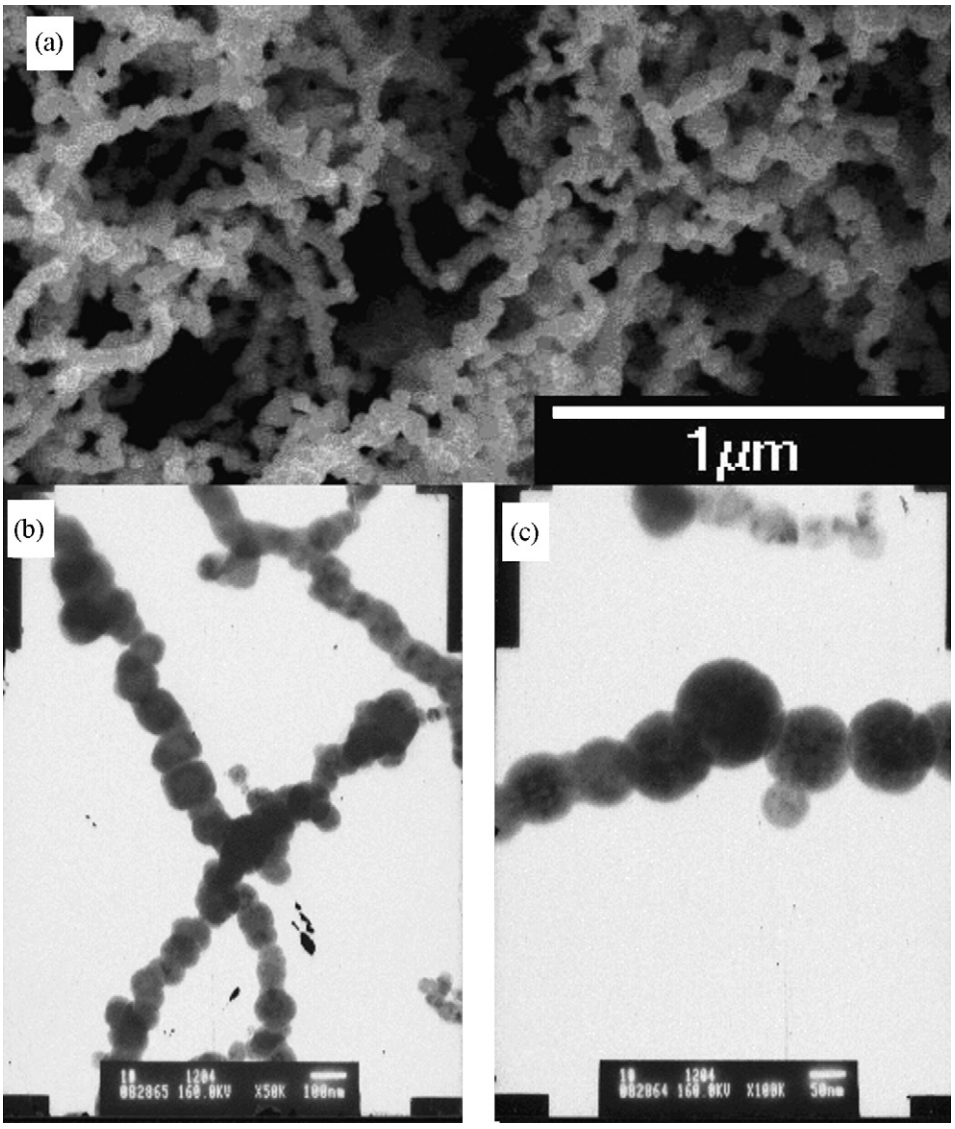


Fig. 1. The SEM (a) and TEM (b, c) images of $Fe@Fe_2O_3$.

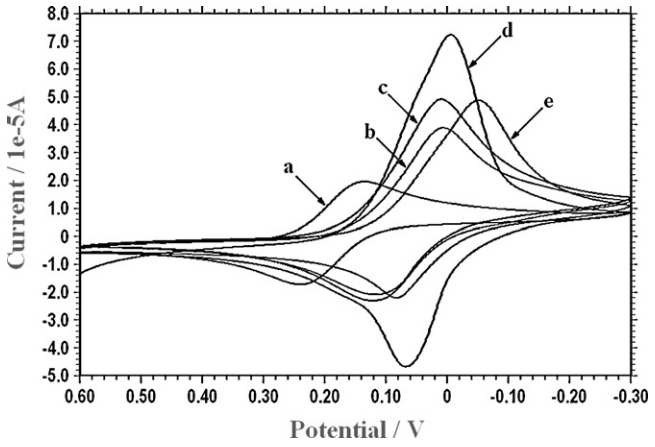


Fig. 2. CVs of 1.0 mM $[Fe(CN)_6]^{3-/4-}$ (1:1) in 0.1 M KCl at different electrodes. (a) Bare GCE; (b) PDDA/GCE; (c) $Fe@Fe_2O_3$–MCNTs/PDDA/GCE; (d) PDDA/$Fe@Fe_2O_3$–MCNTs/PDDA/GCE; (e) DNA/PDDA/$Fe@Fe_2O_3$–MCNTs/PDDA/GCE. Scanning rate: 100 mV s^{-1}.

very easily be oxidized and accordingly aroused an increment of the catalytic current (Eqs. (5)–(7)) [17,18].

$$\text{ds-DNA} \xrightarrow{^{\bullet}OH} \text{DNA(guanine)} \quad (5)$$

$$Ru(NH_3)_6^{3+} + \text{DNA(guanine)} \rightarrow Ru(NH_3)_6^{2+} + \text{DNA(guanine}^{+}) \quad (6)$$

$$Ru(NH_3)_6^{2+} = Ru(NH_3)_6^{3+} + e^{-} \text{ at electrode} \quad (7)$$

3.3. The reproducibility, stability and regeneration of the biosensor

In order to investigate the reproducibility of the biosensor, a batch of seven modified electrodes was fabricated and used to detect the DNA damage. Using 20 mM $Co(phen)_3^{3+}$ as electrochemical indicator, seven ΔI_ps were obtained after the seven electrodes had been treated by the E-Fenton reaction for 30 min. The relative standard deviation (RSD) of seven ΔI_ps was 3.45%, which showed that the biosensor had good reproducibility. The long-term stability of the biosensor was also evaluated, and the experiments showed that ΔI_ps between a new fabricated biosensor and a stored biosensor in the air for 15 days at room temperature had not evident difference. The mean deviation of ΔI_p was less than 5% ($n = 5$). The

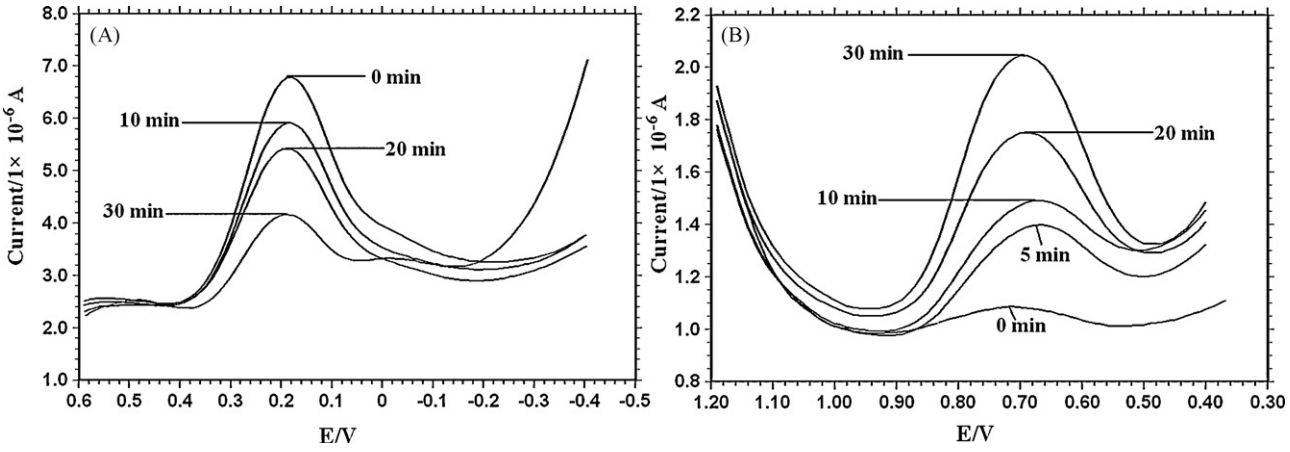


Fig. 3. Differential pulse voltammograms of DNA/PDDA/Fe@Fe_2O_3–MCNTs/PDDA/GCE in 0.1 M pH 5.5 PBS containing 20 mM $Co(phen)_3^{3+}$ (A) or 50 mM $Ru(NH_3)_6^{3+}$ (B) after the electrode was treated by E-Fenton reaction for different time (as indicated in the figure).

biosensor has good long-term stability. After each detection, the biosensor could be regenerated when it was treated as follows: first, the biosensor was treated at −0.6 V for 1 min. Then it was washed with pH 7.0 PBS containing 0.5% of sodium dodecylsulfate (SDS) solution and ultrapure water in sequence in order to remove the damaged DNA, and casted with 8.0 μL of 2.0 mg mL^{-1} ds-DNA on the electrode surface. Thus, the biosensor could be reused. After this biosensor was used for 11 times, the 90% of the first detection signal was obtained. Therefore, this biosensor had good operational stability.

When the 8.0 μL of ds-DNA was used for the damage detection, the minimal detectable amount of DNA damage was determined by gradually decreasing the concentration of ds-DNA coated on the modified electrode. The DPV current change ΔI_p of $Co(phen)_3^{3+}$ before and after the cathodic treatment for 30 min was used as measurement signal. The results showed that the minimal detectable concentration of ds-DNA damage was 0.02 mg mL^{-1}. So the threshold of DNA damage was 0.16 μg.

3.4. The influence of $V_{Fe@Fe_2O_3}$: V_{MCNTs} and pH value on the performance of the biosensor

Fig. 4 shows the influence of $V_{Fe@Fe_2O_3}$: V_{MCNTs} on the performance of the biosensor for DNA damage induced by the E-Fenton reaction. It could be seen that the DPV current change (ΔI_p) of 20 mM $Co(phen)_3^{3+}$ on the biosensor between before and after the E-Fenton reaction for 30 min increased with the increase of $V_{Fe@Fe_2O_3}$: V_{MCNTs}, and reached the maximum value at $V_{Fe@Fe_2O_3}$: V_{MCNTs} of 10:30 and then declined. At ratio of 0:40 or 40:0, namely, when only Fe@Fe_2O_3 core-shell nanonecklace or MCNTs were existed in the membrane, the ΔI_P was small, indicating the obvious synergistic effect of Fe@Fe_2O_3 core-shell nanonecklace and MCNTs to the DNA damage. The optimal ratio of $V_{Fe@Fe_2O_3}$: V_{MCNTs} was 10:30.

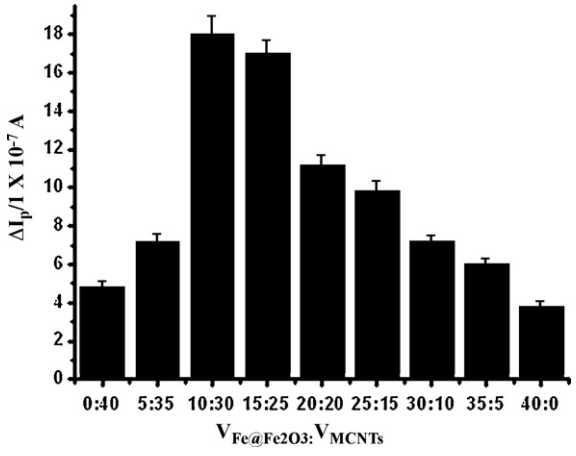


Fig. 4. Effect of the ratio of $V_{Fe@Fe_2O_3}$: V_{MCNTs} in composite on the performance of DNA damage. ΔI_p was the difference of the DPV peak currents of DNA/PDDA/Fe@Fe_2O_3–MCNTs/PDDA/GCE in 0.1 M pH 5.5 PBS containing 50 mM $Co(phen)_3^{3+}$ before and after the treatment by E-Fenton reaction for 30 min.

By studying the change of ΔI_p with the pH value in the range of pH 4.0–7.0, the influence of pH on the DNA damage was also investigated. It was found that the biggest ΔI_p was obtained at about pH 5.0–5.8, which indicated the DNA damage was the most serious in this pH range. Therefore, pH 5.5 was selected as the optimal pH to conduct DNA damage in the E-Fenton reaction.

3.5. The analysis of H_2O_2 and iron ion produced in situ from the biosensing film

The H_2O_2 concentration generated during the E-Fenton reaction was studied by the UV–vis spectrum according to Ref. [19]. The procedure was described in brief as follows: 1.5 mL of substrate solution used in the E-Fenton reaction was taken and added to the mixture solution of 0.75 mL 0.1 M phthalic acid and 0.75 mL aqueous containing 0.4 M KI, 0.06 M NaOH and 1.0×10^{-4} M ammonium molybdate. After it was mixed thoroughly and stood for 2 min, the resulting mixture was analyzed with an UV–vis spectrophotometer by measuring the absorbance at 352 nm. The results are shown in Fig. 5. It was found that no absorption peak appeared at 352 nm at the beginning of E-Fenton reaction (Fig. 5a) indicating no H_2O_2 generated. After this solution had been treated by E-Fenton reaction for 10 min, an absorption peak at 352 nm was obtained and the absorption peak was linear with the time of the E-Fenton reaction, which indicated that H_2O_2 could be produced successively with an almost constant rate during E-Fenton reaction. The concentration of the iron ions generated in E-Fenton reaction was determined by classical 1,10-phenanthroline spectrophotometric method. The procedure was as follows: 1 mL of sample solution treated by cathodic process was mixed thoroughly with 1 mL of 10% hydroxylamine hydrochloride (prepared freshly) and 2 mL of 1.0×10^{-3} M 1,10-phenanthroline solution. After standing for 10 min, the absorption at 510 nm was determined. The results showed that iron ions could also be produced at almost a constant rate from the film during the course of the cathodic treatment. The generation of H_2O_2 and the iron ions with a constant rate was very favorable for a controlled damage of DNA.

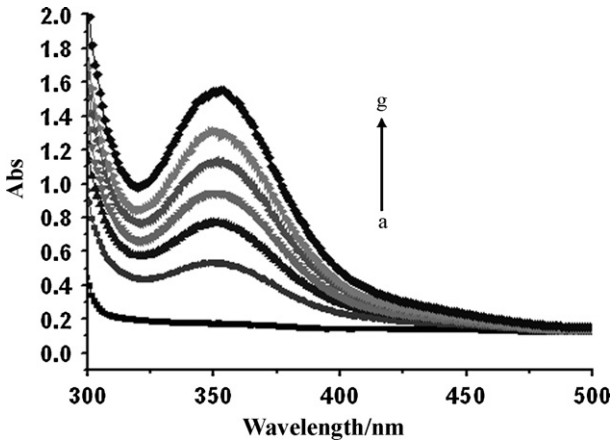


Fig. 5. Concentration changes of H_2O_2 with the reaction time of the E-Fenton reaction. 0 min (a); 10 min (b); 20 min (c); 30 min (d); 40 min (e); 50 min (f); 60 min (g).

The mechanism of the generation of H_2O_2 and iron ion was discussed as follows: during the course of the cathodic treatment, the oxygen dissolved in the solution was adsorbed in the membrane by the porous $Fe@Fe_2O_3$ core-shell nanonecklaces and reached on the surface of the MCNTs. Under the negative potential, the oxygen was reduced to H_2O_2 on the cathode of MCNTs (Eq. (8)). The MCNTs had many advantages, such as good electrical conductivity and mechanical strength, as well as relative chemical inertness to most electrolyte solutions, high surface activity, and a wide operational potential window, so it was ideal material for H_2O_2 electrogeneration according to the literature [20].

$$O_2 + 2H^+ + 2e^- \underset{\text{Cathodic process}}{\overset{\text{MCNTs}}{\longrightarrow}} H_2O_2 \quad (8)$$

$$Fe_2O_3 + 6H^+ \rightarrow 2Fe^{3+} + 3H_2O \quad (9)$$

$$Fe^0 + 2H^+ \rightarrow Fe^{2+} + H_2 \quad (10)$$

$$2Fe^0 + O_2 + 2H_2O \rightarrow 2Fe^{2+} + 4OH^- \quad (11)$$

$$Fe^{3+} + e^- \rightarrow Fe^{2+} \quad (12)$$

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^{\bullet}OH + OH^- \quad (13)$$

$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + {}^{\bullet}HO_2 + H^+ \quad (14)$$

Under the slightly acidic condition, Fe^{3+} and/or Fe^{2+} would be produced via the reactions of the $Fe@Fe_2O_3$ core-shell nanonecklace with H^+ according to Eqs. (9) and (10). Moreover, the Fe^{2+} could also be produced by the reaction of Eq. (11) under aerobic conditions. The iron ions reacted with H_2O_2 to generate hydroxyl radical, the so-called Fenton reaction (Eq. (13)). The hydroxyl radical would attack and induce DNA damage in the film. The Fe^{3+} could be reused via the direct reduction of Fe^{3+} at the electrode (Eq. (12)), or the reaction with H_2O_2 (Eq. (14)) [4,21].

4. Conclusions

In this paper, a new methodology for mimicking the metal-mediated DNA damage pathway *in vivo* was presented. The DNA damage was performed not in the solution containing iron ions and H_2O_2, but in the inner of the sensing film. The iron ions and H_2O_2 were generated *in situ* from the sensing film in a slightly acidic PBS solution. The biosensor could be used to mimic metal-induced DNA damage *in vivo* pathway. Two complementary methods were utilized for monitoring the DNA damage. The method using $Co(phen)_3^{3+}$ could avoid interferences aroused by the oxidizable substances and that using another indicator, soluble $Ru(NH_3)_6^{3+}$, had higher sensitivity than using $Co(phen)_3^{3+}$. This biosensor had not only good performance for DNA damage detection but also the potential for the mechanism understanding of DNA damage induced by oxidative pathways *in vivo*.

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