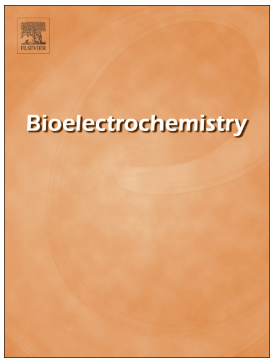

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An impedimetric biosensor for DNA damage detection and study of the protective effect of deferoxamine against DNA damage

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Abstract

The detection and inhibition of DNA damage are of great importance in the prevention and treatment of diseases. Developing a simple and sensitive tool for this purpose would be a chance to monitor the DNA damage and could be helpful in introducing some drugs which can prevent this phenomenon. Here, we report a novel and sensitive electrochemical biosensor based on DNA/Au nanoparticles (AuNPs) modified screen printed gold electrode (DNA/AuNPs/SPGE) to investigate the DNA damage process and also to study the protective behavior of deferoxamine (DFO). The proposed biosensor was fabricated by electrodeposition of AuNPs onto SPGE, followed by chemical immobilisation of thiol-terminated DNA. Cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS) and scanning electron microscopy (SEM) have been used to characterise this biosensor. Hydroxyl radical ($^{\bullet}$OH), which is generated during the Fenton reaction, is responsible for the induced damage to the DNA. EIS technique was applied to monitor the DNA damage, and the increase in charge transfer resistance (R_{ct}) following the DNA damage, was considered as an indicator. Furthermore, the ability of the electrochemical screening system was proved by the investigation of the antioxidant effect of DFO in prohibiting the DNA damage.

Keywords: DNA damage; Au nanoparticles; Screen printed gold electrode; Deferoxamine

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1. Introduction

Reactive oxygen species (ROS) including hydroxyl radical ($^{\bullet}OH$), superoxide radical ($O_2^{\bullet}$) and hydrogen peroxide (H_2O_2), which are produced during normal aerobic metabolism [1] and/or due to exposure to some chemicals in our environment could induce oxidative damage to biological molecules, including DNA, proteins and lipids. Accordingly, it can lead to several diseases such as cancer, diabetes, hypertension and arteriosclerosis [2]. The oxidative damage to DNA is of particular importance, because it is the storage of genetic information, and it also is present in single copies [3].

The Fenton reaction is the source of the most hydroxyl radicals produced in living systems, which includes one-electron transfer from a metal ion (M^n) to the weak O–O bond of H_2O_2, generating the hydroxyl radical and hydroxide anion [4]. Transition metal ions that usually participate in the Fenton reaction are Fe^{2+}, Cu^{2+}, and less common Cr^{2+} [4]. The Fenton-type system is an important mediator of oxidative damage in vivo, and increases the possibility of mutation and chance of cancer [5]. Thus, study of the Fenton-type system is of great interest regarding the decrease of the cancer possibility due to the DNA oxidative damage.

The antioxidants molecules remove the excess ROS, which are produced in human's body, thus making a balance between produced ROS and antioxidant defense system. If overproduction of ROS or insufficient antioxidant defense disturbs this balance, its consequence will be the ROS increase which ends up in oxidative stress [6]. If the DNA damage is not repaired on-time; the induced gene mutation will result in cancer and tumor according to the incomplete DNA replication process [7, 8]. Therefore, an essential way to prevent the DNA damage is using drugs or molecules, which can interact with the excess amount of some metal ions in the body and reduce the amount of ROS formation.

Deferoxamine (DFO), a bacterial hydroxamate siderophore, is one of the widely used drugs in chelating therapy to eliminate excess iron from blood and tissues because of its high affinity for Fe^{3+} chelating [9, 10]. DFO reduces the hydroxyl radical formation and then the oxidative stress reduces because of sequestering redox active iron. The effect of DFO is not only due to chelating but also scavenging a wide range of the oxidant species happens [11, 12]. DFO is a specific Fe^{3+}chelator, it also can bind to the other metal ions such as Cu^{2+} with affinity constant of 10^{14} [13].

Several analytical techniques including fluorescence [14], spectrophotometry [15], chemiluminescence [16, 17], chromatography [18], electron spin resonance [19] and electrochemical techniques [20, 21] have been used to investigate the antioxidant properties of different molecules. Among the mentioned methods, electrochemical techniques [20] are of particular significance, because of their advantages such as fast analyses, high sensitivity, simplicity, inexpensive instrumentation and easy operation. Among different electrochemical approaches, the DNA-based sensors are the most effective one for assessing antioxidants capacity. Several electrochemical DNA-based sensors have been developed for measurement of the antioxidant capacity of different compounds [4, 22-29] which are based on immobilisation of DNA on the electrode surface.

The DNA immobilisation process is a major step affecting on stability, reproducibility and sensitivity of the DNA sensor. Introducing the self-assembled monolayer (SAM) of DNA on the surface by getting the help of the perfect affinity between thiol and gold is the most popular immobilisation way to make stable biosensors. Gold nanoparticles have been widely used in the modification of various electrodes for fabrication of different kinds of biosensors. These nanoparticles offer large specific surface area, strong adsorption ability, excellent conductivity [30-34] and can greatly enhance the amount of immobilised ssDNA on the surface.

In this study, we investigate the antioxidant properties of DFO on the Fenton system-induced DNA damage by an electrochemical screening system based on DNA biosensor. Because of the importance of tandem repeats of d (TTAGGG) sequences in human genome structure and many specific cancers, this sequence is chosen as a model of human telomeric DNA in this study. To the best of our knowledge, the impedimetric investigation of the DNA damage by AuNPs-SPGE has not reported yet. Moreover, there is no report regarding the investigation of DFO antioxidant properties by electrochemical DNA sensor.

2. Experimental

2.1. Chemicals

The sequence of the single-stranded human telomeric DNA (ssDNA), which purchased from MWG-Biotech Company, (Germany), is as follow:

SH-5′ GGG TTA GGG TTA GGG TTA GGG 3′ (human telomeric DNA)

The SH-DNA stock solution (100 μM) was prepared using water and kept in the freezer. Before use, as a precaution, the SH-DNA stock solution was treated with Tris 2-carboxyethyl phosphine (TCEP) to cleave the disulfide bonds of oligo dimers eventually acquired by oxidative coupling of two SH-DNA molecules. For this purpose, 1.0 mL of the 100 μM stock solution of DNA and 1.0 mL of 0.01 M TCEP were mixed in a tube, and the reaction was allowed to proceed for 60 min at the ambient temperature. Then, the solution was diluted to 1.0 μM with 20.0 mM Tris–HCl buffer solution (TBS) (pH 7.00) containing 20.0 mM NaCl.

Desferal (deferoxaminemesylate, often mentioned as deferoxamine, desferoxamine or desferrioxamine), TCEP, 6-mercapto-1-hexanol (MCH), Tris–HCl, Na_2HPO_4, NaH_2PO_4, NaCl and NaOH were supplied from Sigma. Tetrachloroauric (III) acid trihydrate ($HAuCl_4 \cdot 3H_2O$), sulfuric acid (H_2SO_4), hydrogen peroxide (H_2O_2, 35% w/v), $CuSO_4.5H_2O$, ascorbic acid (AA), potassium ferricyanide and potassium ferrocyanide were acquired from Merck. All solutions were prepared using reagent grade chemicals and deionized and sterilized water. All the experiments were carried out at room temperature.

2.2. Instrumentation and methods

All Electrochemical measurements were performed with AUTOLAB, PGSTAT 30 (EcoChemie, Netherlands) equipped with GPES 4.9 and FRA software. The screen-printed gold electrodes (SPGE Florence, Italy) were made up of three main components: a gold working electrode (WE), a gold auxiliary electrode and a silver pseudo reference electrode. Scanning electron microscopy (SEM) micrographs were recorded with SEM-KYKY-EM 3200, China.

Cyclic voltammetry (CV) was employed for electrochemical characterisation of different modified electrodes in the potential range of −0.6 to 0.8 V with a scan rate of 50 mV s^{-1} in 0.1 M PBS (pH 7.0) containing 1.0 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (1:1).

The EIS measurements were performed at a polarization potential of 0.17 V within the frequency range of 10 kHz - 0.1 Hz using an amplitude of 0.005 V in the presence of 1.0 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ as a redox probe in 0.1 M PBS (PH 7.0). The obtained Nyquist plots were fitted to an equivalent circuit to extract the value of charge transfer resistance (R_{ct}). All the measurements were carried out at least three times, and the error bars were shown in the following figures.

2.3 Preparation of DNA biosensor

Before modifying the SPGE, the WE was pretreated by multiple-pulse amperometry in a solution containing 0.5 M H_2SO_4 and 10 mM KCl [35]. Triple-potential pulse sequence: −0.3 V for 0.30 s; 0.0 V for 0.30 s and +1.0 V for 0.15 s (150 cycles) was applied. Then, AuNPs were electrodeposited on the WE surface using chronoamperometry by applying the step potential of − 0.23 V for 300 s in 0.5 mM of the $HAuCl_4$ aqueous solution. Before immobilisation of thiol-terminated DNA, the modified electrode was rinsed with water. The AuNPs modified WE surface was then exposed to the 10 μL of 1.0 μM DNA solution and permitted to proceed overnight. During this time, the electrodes were kept in a petri dish in the wet chamber to prevent evaporation of the solution. The thiol-terminated DNA was covalently linked to the surface of the AuNPs/SPGEs via Au–S binding. After rinsing with water, the DNA modified WEs surfaces were exposed to the MCH solution (10 μL, 1.0 mM MCH in water) for one hour to remove the nonspecific adsorbed DNA. These modified SPGEs were designated as DNA/AuNPs/SPGEs. Both the DNA and MCH solutions were manually drop casted on the WEs surfaces. The ability of the developed biosensor for detection of the DNA damage induced by the Fenton reaction was assessed in the absence and presence of DFO. The overall process was summarized in Scheme 1.

"Here Scheme 1"

2.4. Procedure

The DNA damage was conducted by immersing the fabricated DNA/AuNPs/SPGE in the damaging solution (1.0 mL of TBS (pH 7.0 containing (2.5×10^{-8} M $CuSO_4$, 2.5×10^{-4} M H_2O_2 and 1×10^{-6} M AA)) which were optimized according to the literature [36, 37] under stirring (100 rpm) for 60 min. To investigate the DNA damage, EIS measurements were performed before and after immersion of DNA/AuNPs/SPGE in the damaging solution. In the final step of this study, the effect of DFO as an antioxidant against DNA damage was investigated to see whether this antioxidant could act as a protecting agent for DNA, and prevent its damage. For this aim, the degree of DNA damage was evaluated in the presence of different concentrations of DFO and the EIS spectra were recorded. Each measurement was repeated at least three times. In order to eliminate electrode-to-electrode or film-to-film

variation, the normalized value of R_{ct} (Eq. (1)) was adopted instead of the absolute R_{ct} as the measurement signal for repetitive experiments.

$$\frac{\Delta R}{R} = (R_{ct,t} - R_{ct,0})/R_{ct,0} \quad (1)$$

Where $R_{ct,0}$ and $R_{ct,t}$ are the resistance charge transfer value before and after immersion of DNA/AuNPs/SPGE in the damaging solution, respectively. Moreover, control experiments were performed by immersing the DNA biosensor in a solution containing one of the $CuSO_4$ (2.5×10^{-8} M), H_2O_2 (2.5×10^{-4} M), or blank solution (1 mL of TBS (pH 7.0) and mixture solutions.

3. Results and discussion

3.1 Morphology characterisation of the fabricated biosensor

Fig. 1 shows the SEM images of bare SPGE (A), AuNPs/SPGE (B), and DNA/AuNPs/SPGE (C). The SEM micrograph of AuNPs/SPGE indicates that a uniform coverage of AuNPs was formed on the surface with particles sizes of 20-60 nm which can lead to an increase in effective surface area [38, 39]. After the tiolated DNA immobilisation on the surface of AuNPs/SPGE, the surface morphology was changed (Fig. 2C), indicating the uniform DNA assembly on the AuNPs/SPGE surface.

"Here Fig. 1"

3.2 Electrochemical characterisation of stepwise sensor fabrication process

Electrochemical characterisation of the stepwise DNA sensor fabrication process was carried out via CV and EIS techniques. Fig. 2A shows the cyclic voltammograms of bare SPGE, AuNPs/SPGE and DNA/AuNPs/SPGE in 0.1 M PBS (pH 7.0) containing 1.0 mM $[Fe(CN)_6]^{3-/4-}$ redox couple. A couple of redox peaks can be observed at bare SPGE (a) with a cathodic peak current (I_{pc}) of -11.6 μA, an anodic peak current (I_{pa}) of 11.4 μA, and peak-to-peak potential separation ($\Delta E_p = E_{pa} - E_{pc}$) of 0.44 V (curve a). When AuNPs deposited onto the SPGE surface, current responses were obviously increased (I_{pc} = -17. 2 μA and I_{pa} = 20.8 μA), and ΔE_p was significantly decreased to 0.17 V, (curve b). This observation is probably due to the large surface area and excellent conductivity of AuNPs [32, 40, 41]. After

immobilisation of thiol terminated DNA onto the AuNPs/SPGE surface, the current responses were significantly decreased (I_{pc} = -10. 7 μA and I_{pa} = 10.41 μA), and ΔE_p was increased to 0.64 V (curve c). This is probably attributed to the electrostatic repulsion between negatively charged phosphate groups of DNA and $[Fe(CN)_6]^{3-/4-}$ that hinder the diffusion of $[Fe(CN)_6]^{3-/4-}$ to the electrode surface. These results show that the covalent immobilisation of thiol terminated DNA has been successfully performed.

"Here Fig. 2"

The EIS is a non-destructive, less time-consuming steady-state technique that is capable to probe the relaxation phenomena over a range of alternative current frequencies. The Nyquist plots of EIS include a linear section at lower frequencies ascribed to a diffusion-limited process, and a semicircle part at higher frequencies related to the electron-transfer limited process. The increase in the semicircle diameter reflects the increase of the interfacial charge-transfer resistance (R_{ct}) [42, 43]. Fig. 2B displays the electrochemical impedance spectra corresponding to the stepwise modification processes of the SPGE in 0.1 M PBS (pH 7.0) containing 1.0 mM $[Fe(CN)_6]^{3-/4-}$ redox couple. Fig. 2B Curve a, represents the Nyquist plot of EIS for bare SPGE, with the R_{ct} of about 5 kΩ. After modification of SPGE with AuNPs, the R_{ct} value was significantly decreased to 2 kΩ, (curve b). The reason for this observation may be related to the promotion of the electron transfer rate between $[Fe(CN)_6]^{3-/4-}$ and electrode. The self-assembly layer of DNA on the AuNPs/SPGE surface effectively repulses the $[Fe(CN)_6]^{3-/4-}$ anions and thus eventuates to huge enhanced charge transfer resistance (25 kΩ, curve c). Modeling of the impedance data was realized according to the presented equivalent circuit in the inset of Fig. 2B, where R_{ct} is the charge transfer resistance, CPE is the constant phase element, R_s is the solution resistance and Z_w is the Warburg impedance.

3.3 Impedimetric detection of the DNA damage

The DNA damage sensing was performed via the DNA sensor based on EIS detection, which is a tool that transduces the alterations in the interfacial properties between the electrode and the electrolyte created by DNA damage to an electrical signal. Fig.3 (A) displays electrochemical impedance spectra of DNA/AuNPs/SPGE before (a) and after

immersion in the damaging solution (b), for 60 min under stirring. As can be seen, following immersion of DNA/AuNPs/SPGE in the damaging solution, the R_{ct} was dramatically increased from (25 kΩ, curve a) to (50 kΩ, curve b). The possible reason for this result is that $^{\bullet}OH$ cause DNA oxidative damage, creating different kind of modifications at DNA level including, strand breaks, base and sugar lesions, DNA-protein cross-linking and base-free sites which leads to the difficulty of redox reactions of $[Fe(CN)_6]^{3-/4-}$, and increasing the R_{ct} value [44-48]. This behavior can be used to detect the DNA damage using EIS monitoring. Fig. 3B shows the obtained results from the control experiments. As can be seen, no significant changes in the R_{ct} values were observed after immersion of the sensor in the individual solutions of the $CuSO_4$ (b), H_2O_2 (c) or blank solution (a) in comparison with mixture solutions; ($CuSO_4$ + AA) (d), ($CuSO_4$ + H_2O_2) (e) and ($CuSO_4$ + H_2O_2 + AA) (f). These results demonstrate as follows: ascorbic acid is an antioxidant, but it also can act as a pro-oxidant reagent in the presence of metal ions [49, 50]. As it is known, ascorbate is the prevalent form of ascorbic acid in physiological pH [51]. It can reduce metal ions and acts as a source of H_2O_2 following its auto oxidation by oxygen [52]. These reduced form of metal ions can reduce H_2O_2 to generate $^{\bullet}OH$ which cause DNA damage (d) [53]. Besides, the reaction between Cu^{2+} ions with H_2O_2 (e) produces $^{\bullet}OH$, which creates DNA damage [54]. As can be seen, Cu^{2+}/H_2O_2/AA system (f) showed much greater damage than Cu^{2+}/H_2O_2 system (e) according to following mechanism. (Eqs. (2)-(4)) [55].

$$Cu^{2+} + AH^{-} \rightarrow Cu^{+} + A \qquad (2)$$

$$Cu^{+} + H_2O_2 \leftrightarrow Cu^{2+} + {}^{\bullet}OH + OH^{-} \qquad (3)$$

$$Cu^{2+} + AH^{-} \rightarrow Cu^{+} + A \qquad (4)$$

In the Fenton reaction (3), the reduced form of copper, Cu^{1+}, reacts with H_2O_2 to produce the oxidized form of copper and hydroxyl radical. In the presence of ascorbate, the generated oxidized form of copper by the Fenton reaction can be reactivated (4), thus allowing additional $^{\bullet}OH$ to be generated [55].

"Here Fig. 3"

3.4. Influence of incubation time

The incubation time is an important factor affecting on the DNA damage. The effect of incubation time was investigated by placing of DNA/AuNPs/SPGEs in the damaging solution under the stirring condition between 0 and 90 min, followed by EIS measurements. Fig.4

indicates that the response was increased for the first 60 min, and then was reached a plateau for the prolonging time. Hence, 60 min was selected as a compromise between the damage degree and work efficiency.

"Here Fig. 4"

3.5 Evaluation of the protective effect of deferoxamine on DNA against damage

In order to inspect the protective effect of DFO on DNA damage, EIS technique was applied. Fig. 5A represents the Nyquist plots of the DNA/AuNPs/SPGEs before (a), and after immersion in the damaging solution in the absence (b) and the presence of DFO (c). As can be seen, no significant increase in the R_{ct} value was observed after immersion of DNA/AuNPs/SPGE in the damaging solution containing DFO. This result illustrates that DFO can prevent DNA damage due to its antioxidant activity. It can be proposed that the antioxidant activity of DFO is related to its copper chelating capability. Therefore, after chelate formation, the complex form of Cu^{2+} ions /DFO has less ability for inducing ascorbate oxidation and leads to stop of $^{\bullet}OH$ formation pathway in its first step (Eq. (2)), [56]. In addition, the protecting effect of different concentration of DFO (2.5×10^{-10} - 7.5×10^{-7} M) against DNA damage was studied and the results were depicted in Fig. 5B. As seen in this figure, the measurement signal , $\left(\frac{\Delta R}{R}\right)$, was decreased as DFO concentration was increased, and the DNA damage was completely prevented in 7.5×10^{-7} M of DFO. All these results show that DFO has the prevention capability of the DNA damage in this system and the proposed DNA biosensor can recognize this effect.

"Here Fig. 5"

3.6 Reproducibility and stability of DNA/AuNPs/SPGE

The reproducibility of DNA/AuNPs/SPGE was examined by recording the R_{ct} value for six DNA sensors, which had been prepared in the same procedure. The relative standard deviation was obtained as 4.13% revealing that this DNA sensor has suitable reproducibility.

The stability of the fabricated DNA sensor was also evaluated over a three-week period. During this time, DNA/AuNPs/SPGE was kept in ultra-pure water at 4 $^{\circ}$C and the R_{ct} value

was measured intermittently (every 3–7 days). The sensor response did not show any substantial change indicating that DNA/AuNPs/SPGE has reasonable stability.

A comparison between previously published literature and this research was listed in table 1. Our new sensor studied label free detection of the DNA damage induced by Fenton system using the EIS technique. EIS has some advantages compared to other electrochemical methods, including ease of signal quantification, high sensitivity and non-destructive [43]. This sensor has the ability to detect both the DNA damage and the antioxidant effect of DFO in preventing the DNA damage. Moreover, this method is simple, fast, without electroactive indicator requirement, and can be used at biological pH for damage induction and detection. However the covalent attachment method used for DNA immobilisation suffers from requirement of modified DNA or additional cross-linking reagent compared with other physical DNA immobilisation methods [57]. But one of the main advantages of this method which convinced us to utilize it, is that it can create more stable sensors with high DNA stability compared with the physical adsorption methods. Because, desorption of the adsorbed DNA can be happen by changes in some parameters such as pH, ionic strength and temperature in the physical adsorption methods [57].

"Here Table 1"

4. Conclusion

In summary, this work illustrated label free detection of the DNA damage based on DNA/AuNPs/SPGE using EIS technique. The fabricated biosensor showed a successful function for evaluation of both the DNA damage and the antioxidant properties of DFO against DNA damage. Preparation and detection protocols are simple, low cost, required a short time for analysis and can be easily adapted for the detection of the DNA damages induced by different damaging systems. These low cost miniaturized disposable electrodes due to the possibility of combining portable equipment are promising devices for the rapid and inexpensive evaluation of antioxidant capacity of different molecules.

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Table 1. A comparison study between different electrochemical approaches for detection of the Fenton induced DNA damage.

Sensor	Method	Damaging reagent	Signal	DNA attachment method	Long term Stability	Ref.
CS/ds-DNA/Ag-PGL/GCE	LSV	Fe^{2+}/H_2O_2	Change in I_{pa} of $Ru(NH_3)_6^{3+}$	adsorption	15 days	[28]
DNA–XOD/GCE	SWV	xanthine/$FeSO_4$	Change in I_{pa} of $Co(bpy)_3^{3+}$	adsorption	7 days	[46]
DNA–XOD/GCE	SWV	$FeSO_4$/glucose	Change in I_{pa} of $Co(bpy)_3^{3}$	adsorption	_	[47]
dsDNA/GCE	SWV	Fe^{2+}/EDTA/H_2O_2	Change in I_{pa} of DNA bases	adsorption	_	[48]
(MWCNTs/PDDA/ ds-DNA)$_2$/ PGE	DPV	Cr(VI)/GSH/H_2O_2	Change in I_{pa} of MB	LBL	_	[58]
(PDDA/dsDNA)$_3$/ GCE	CV	V_2O_5nanobelts/HCl/ H_2O_2	Change in I_{pa} of $Ru(bpy)_3^{2+}$ and I_{pc} of $Co(phen)_3^{3+}$	LBL	30 days	[59]
DNA/GCE	SWV	Fe^{2+}/ H_2O_2	Change in I_{pa} of $Co(bpy)_3^{3+}$	adsorption	_	[60]
DNA/AuNPs/SPGE	EIS	$CuSO_4$/ H_2O_2/AA	Change in R_{ct}	Covalent attachment	21 days	This work

PDDA: poly (diallyldimethylammonium chloride); PGE: Pencil graphite electrode; GSH: Glutathione (γ-l-glutamyl-l-cysteinyl-glycine); MB: Methylene blue; GCE: Glassy carbon electrode; CS: Chitosan; PGL: Poly l-glutamic acid; LSV: linear sweep voltammetry, I_{pa}: anodic peak current, I_{pc}: cathodic peak current, LBL: Layer by layer

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Figures caption

Scheme 1 Schematic representation of entire procedure of DNA sensor fabrication and DNA damage detection.

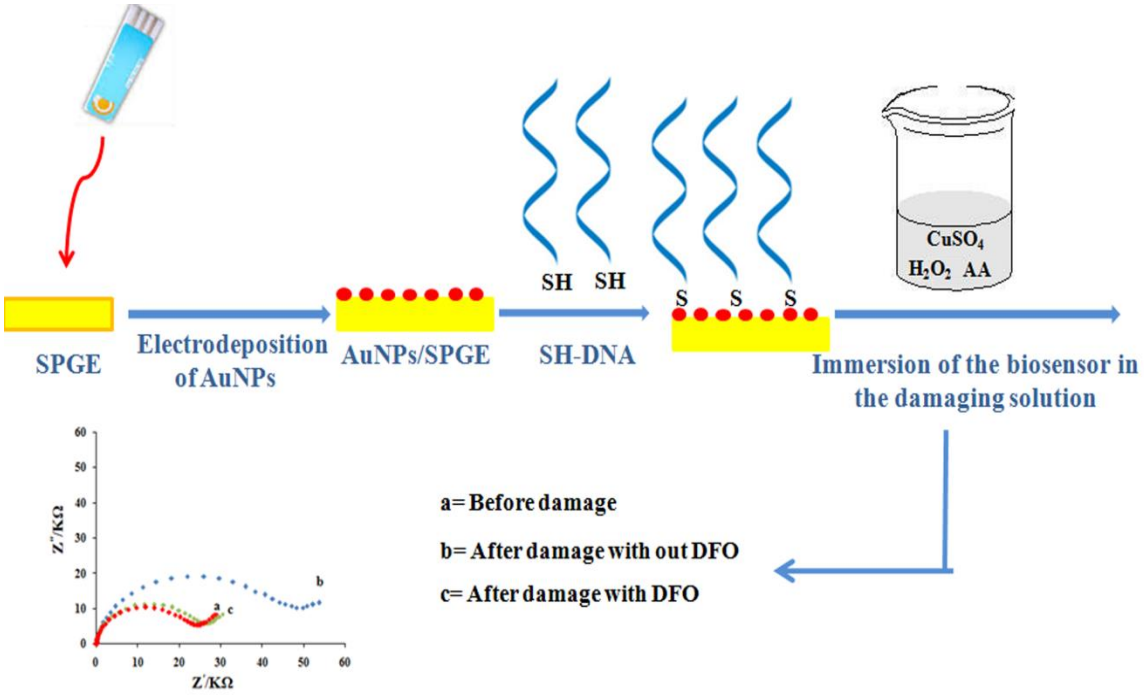
Fig. 1. The SEM images of bare SPGE (A), AuNPs/SPGE (B) and DNA/AuNPs/SPGE (C).

Fig. 2 (A). Cyclic voltammograms of bare SPGE (a), AuNPs/SPGE (b) and DNA/AuNPs/SPGE (c) in 0.1M PBS (pH 7.0) containing 1.0 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (1:1) at scan rate of 50 mVs^{-1}. **(B)** The Nyquist plots of bare SPGE (a), AuNPs/SPGE (b) and DNA/AuNPs/SPGE (c) in 0.1 M PBS (pH 7.0) containing 1.0 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (1:1) redox couple.

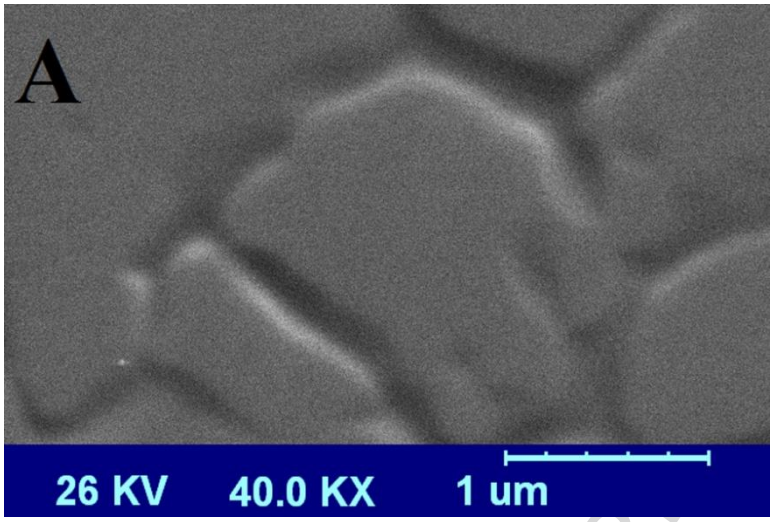
Fig .3 (A) Nyquist plots of DNA/AuNPs/SPGE (a) before and (b) after immersion in the damaging solution, in 0.1 M PBS (pH 7.0) containing 1.0 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (1:1). **(B).** The $\Delta R/R$ values for DNA/AuNPs/SPGE after incubation with TBS (pH 7.0) containing blank (a), 2.5×10^{-8} M $CuSO_4$ (b), 1×10^{-4} M H_2O_2 (c), 2.5×10^{-8} M $CuSO_4$ + 1×10^{-6} M AA (d), 2.5×10^{-8} M $CuSO_4$ + 1×10^{-4} M H_2O_2 (e) and 2.5×10^{-8} M $CuSO_4$ + 1×10^{-4} M H_2O_2 + 1×10^{-6} M AA (f).

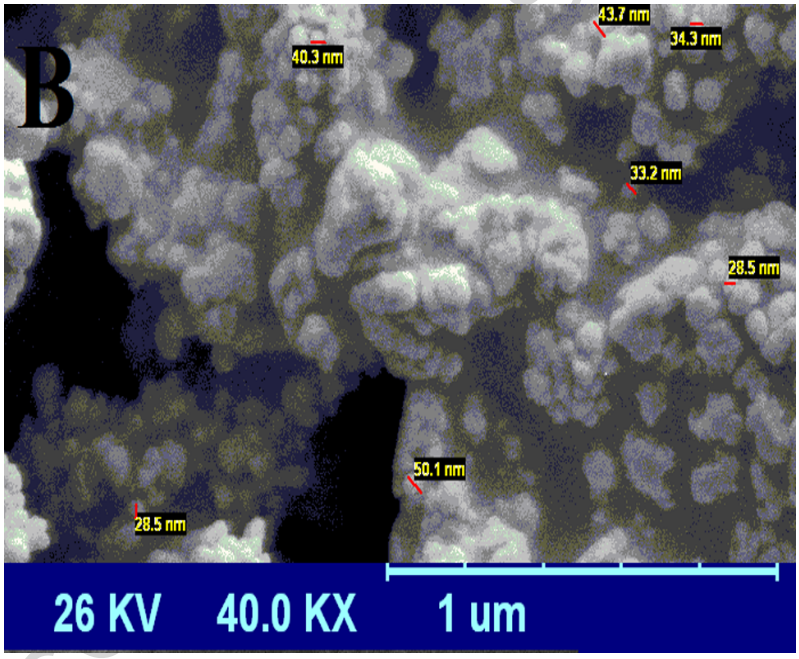
Fig. 4. Dependence of the DNA damage degree on the incubation time.

Fig. 5 (A). The EIS spectra of DNA/AuNPs/SPGE before (a) and after immersion in the damaging solution in the absence (b) and the presence of 7.5×10^{-7} M DFO (c), in 0.1 M PBS (pH 7) containing 1.0 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (1:1). **(B).** The dependence of $\Delta R/R$ to different concentrations of DFO (0.0, 0.25×10^{-9}, 0.50×10^{-9}, 1.0×10^{-9}, 2.5×10^{-9}, 5.0×10^{-9}, 7.5×10^{-9}, 1.0×10^{-8}, 2.5×10^{-8}, 5.0×10^{-8}, 1.0×10^{-7}, 2.5×10^{-7}, 5.0×10^{-7}, 7.5×10^{-7} M). The illustrated error bars represent the standard deviations of three repeated measurements.



Scheme 1





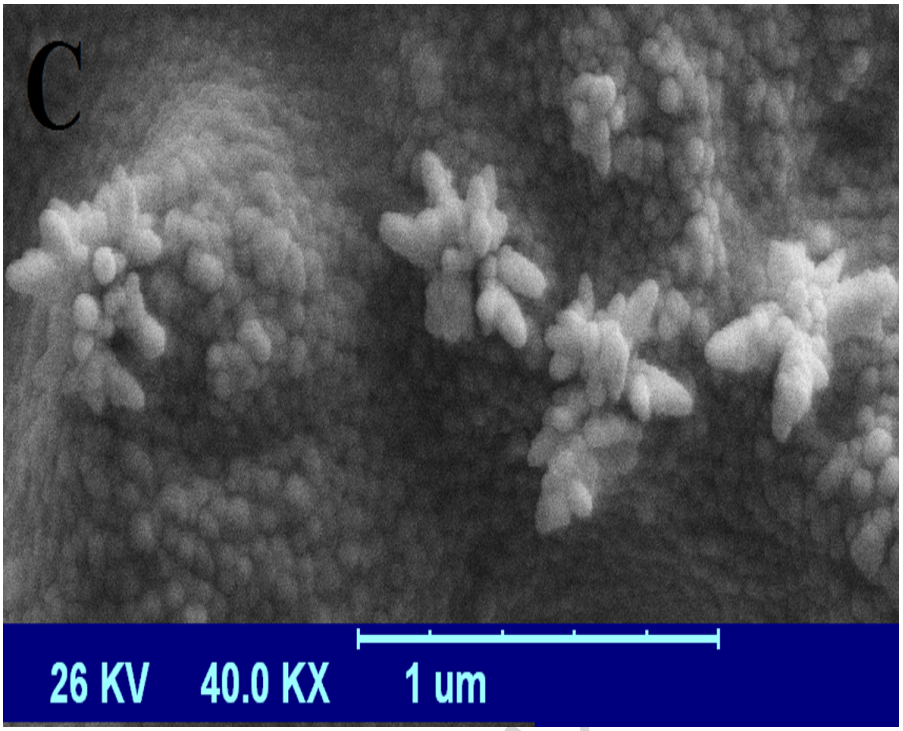


Fig. 1

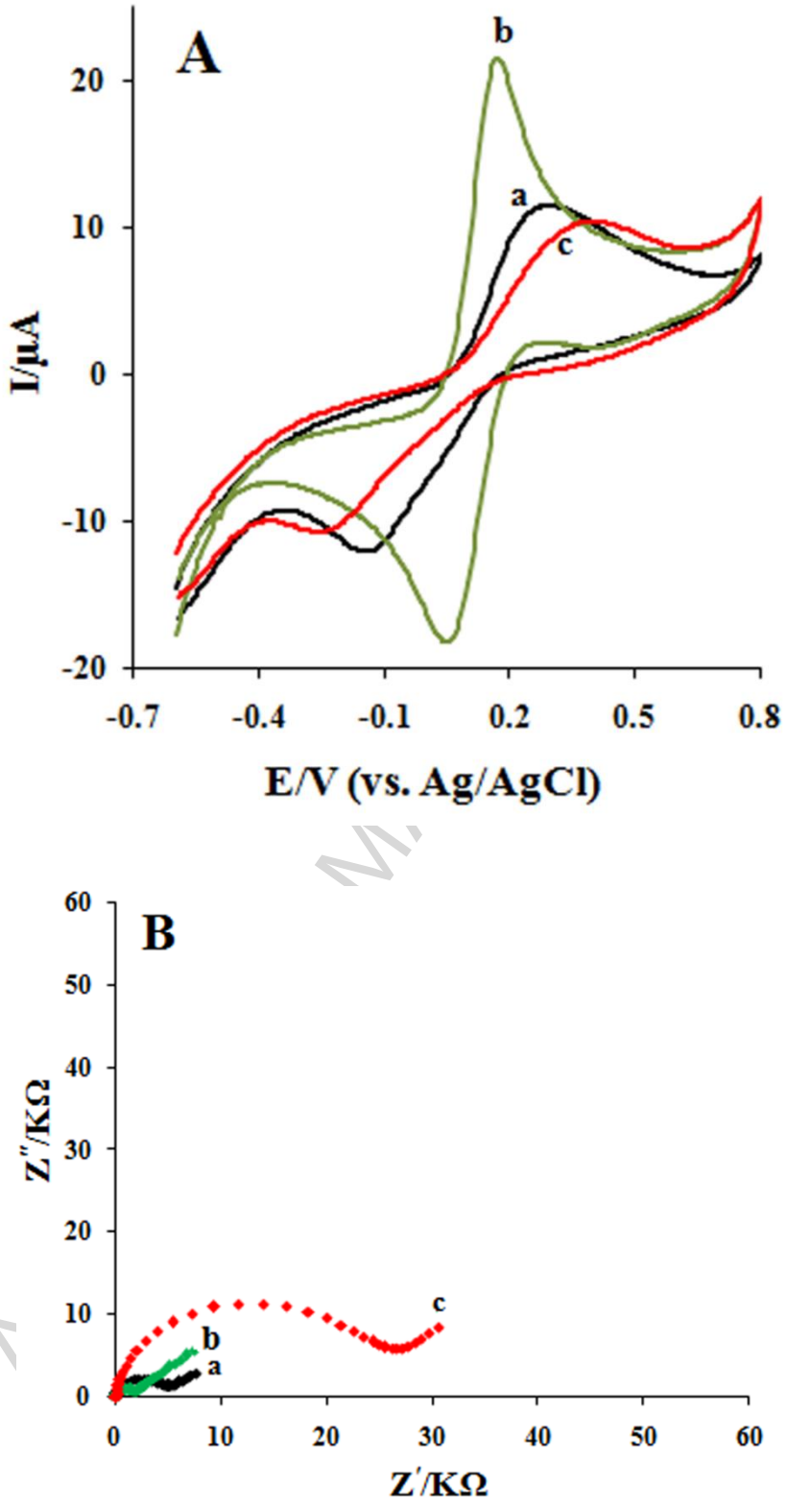


Fig. 2

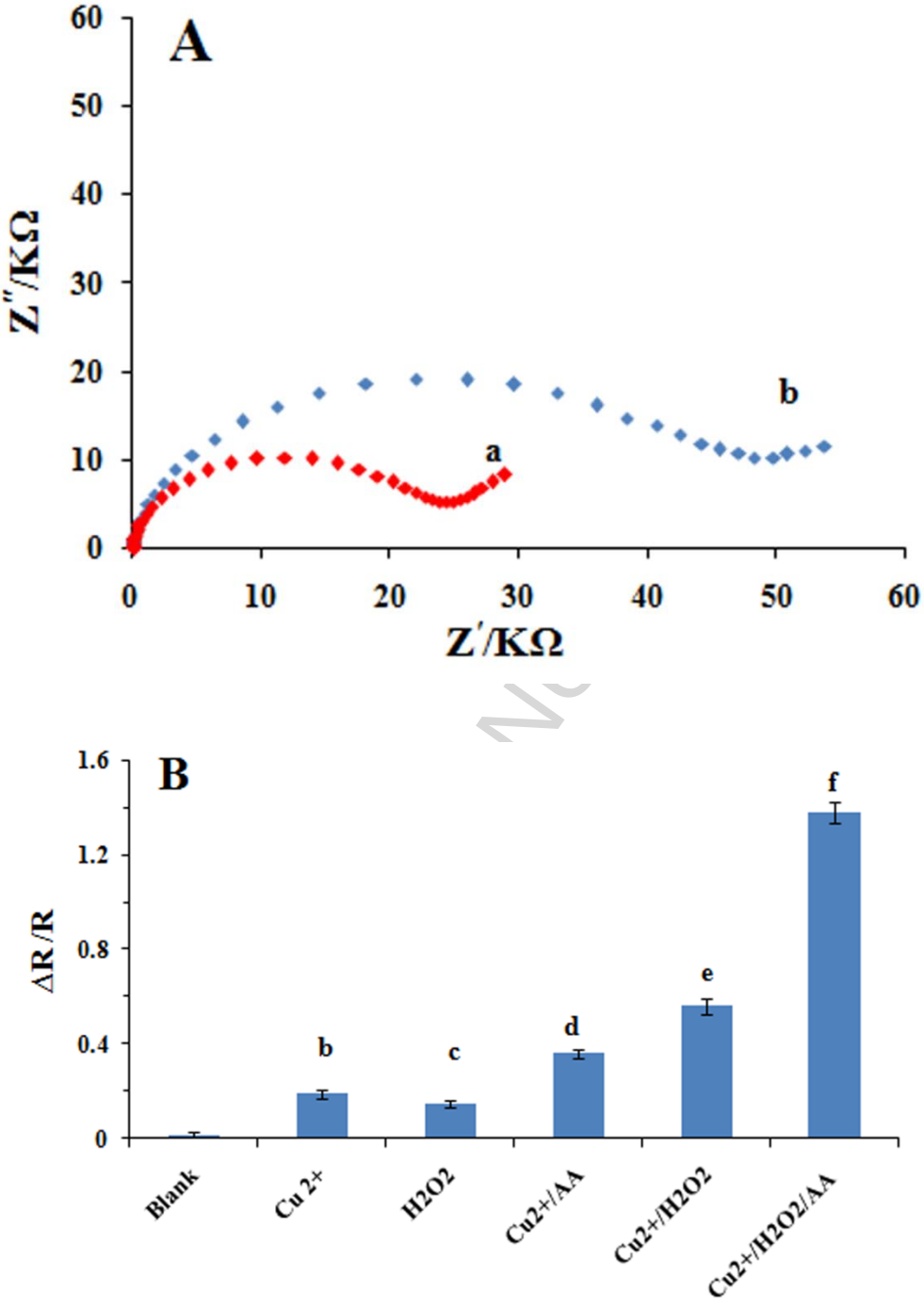


Fig. 3

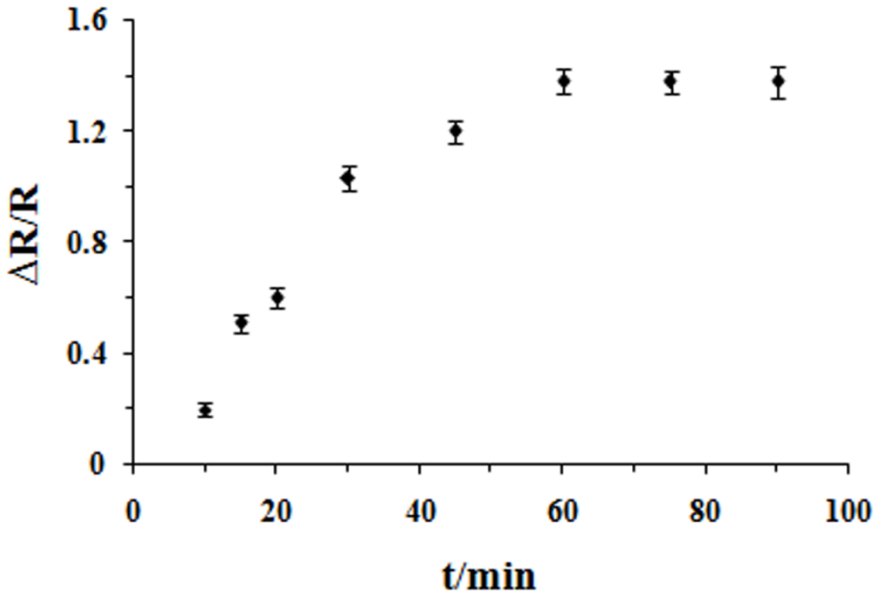


Fig. 4

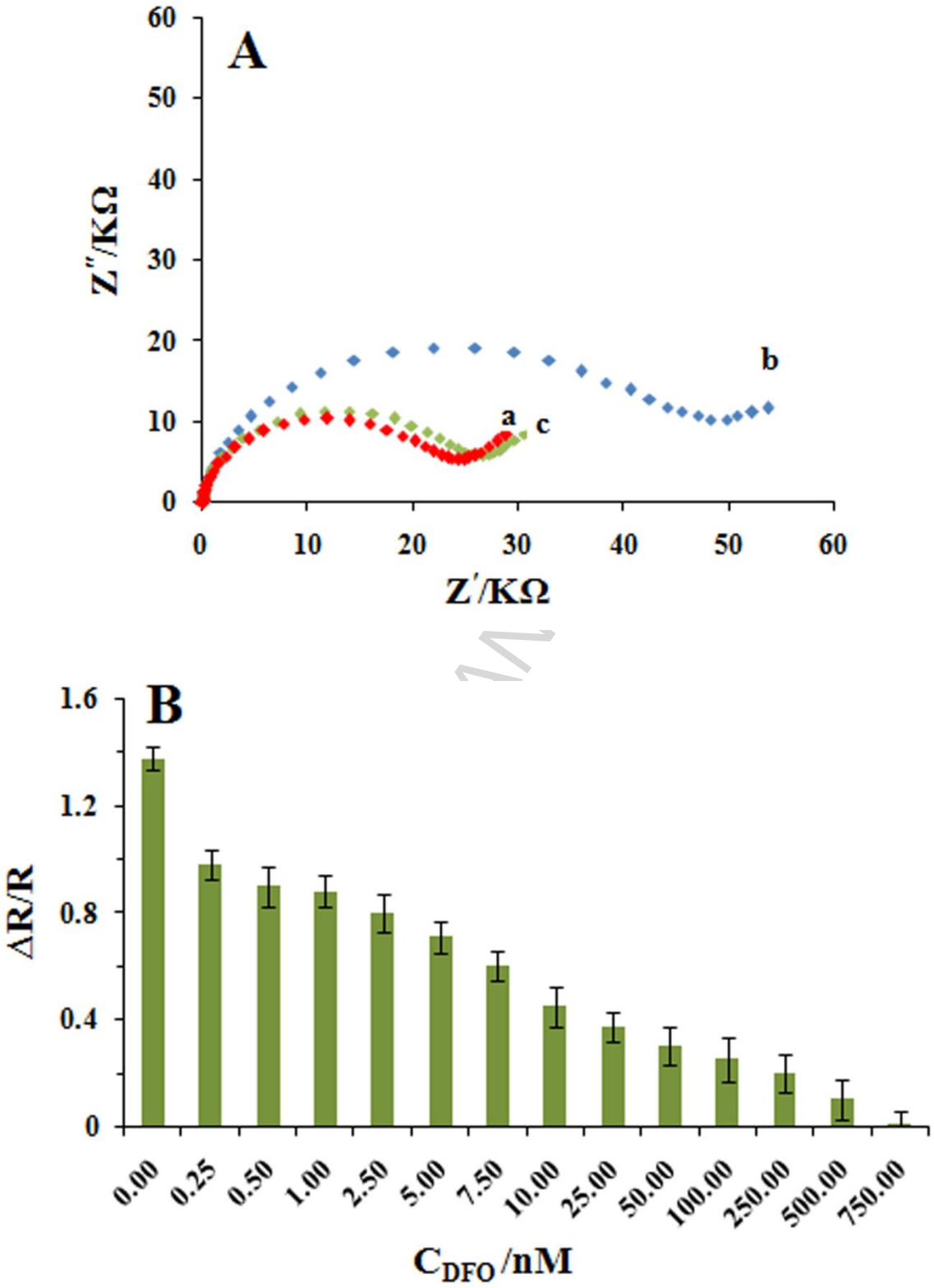
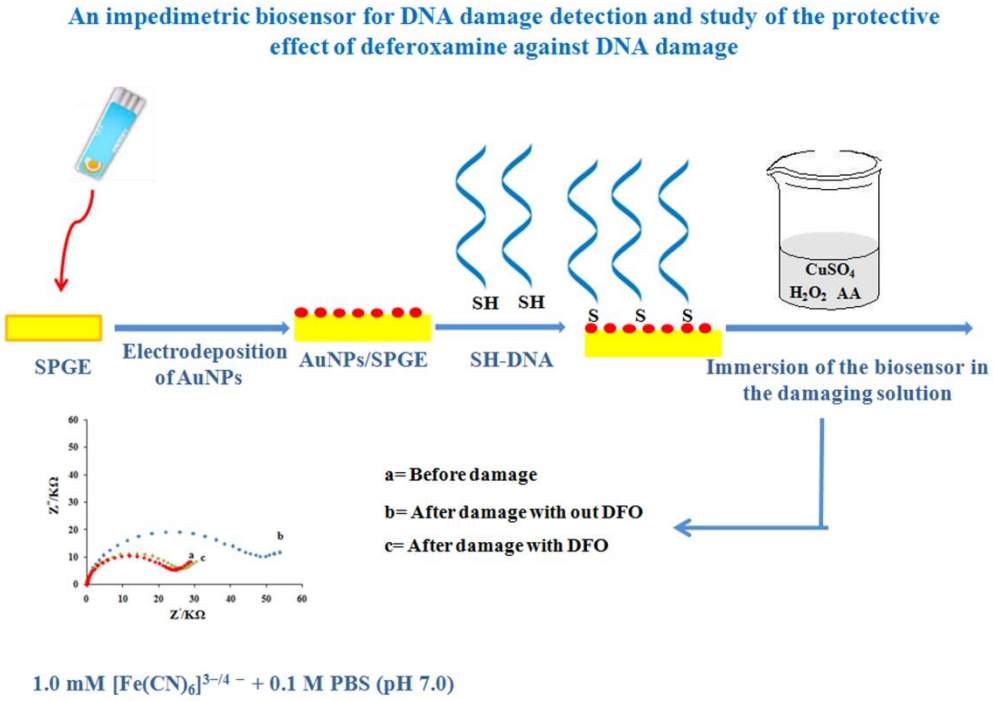


Fig. 5

Graphical Abstract



Highlights:

- The DNA/AuNPs/SPGE has been fabricated as a new biosensor.
- The biosensor was employed for direct detection of DNA damage using EIS technique.
- DNA damage was induced by $^{\bullet}OH$ generated from Fenton reaction.
- The ability of deferoxamine as an antioxidant for preventing DNA damage was studied.