

Fabrication of CdTe@SiO₂ nanoprobe for sensitive electrogenerated chemiluminescence detection of DNA damage

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Detection of DNA damage is significant for the evaluation of genotoxicity of new chemicals in the early stages of its development. An electrogenerated chemiluminescence (ECL) biosensor was fabricated to detect specific sequences of DNA by using CdTe@SiO₂ as nanoprobe for signal amplification. This DNA biosensor was constructed by self-assembly of an aminated capture DNA on the glass carbon electrode. DNA detection was realized by outputting a remarkable ECL signal of the CdTe@SiO₂ labeled probe DNA. When the target DNA was introduced into the system, it was complementary to the probe DNA at the one-half-segment and complementary to the capture DNA at the other half-segment, resulting in the formation of a stable duplex complex. As a result, the CdTe@SiO₂ labeled probe was proximate to the electrode surface and the ECL was observed. This DNA biosensor was proved to have a low detection limit (0.03 nM) and a wide dynamic range (from 0.1 nM to 2 μM). Most importantly, the sensing system could differentiate the single base mismatched DNA from the complementary DNA. It was successfully applied to study the damage to DNA caused by several genotoxicity chemicals, which was rapid, simple, reliable and sensitive compared to the classical biological methods.

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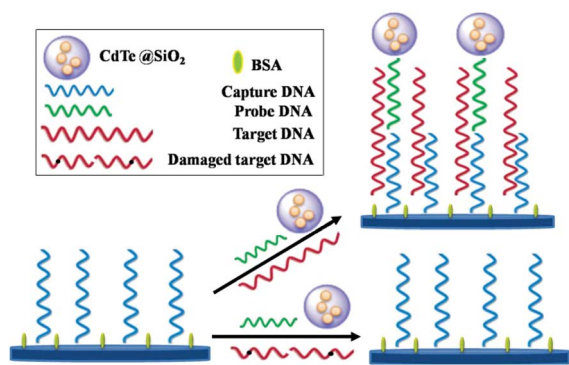
1 Introduction

Most cancer results from the interaction of genetics and the environment. Genetic factors by themselves are thought to explain only about 5% of all cancers. The remainder can be attributed to external “environmental” factors that act in conjunction with both genetic and acquired susceptibility.¹ Environmental factors include different man-made chemicals existing in the workplace, air, water, food supply, drugs and certain types of radiation. These chemicals with affinity to interact with DNA finally give rise to the different types of DNA damage such as oxidation of bases, alkylation of bases, hydrolysis of bases, bulky adduct formation and mismatch of bases. In rapidly dividing cells, unrepaired DNA damage that does not kill the cell by blocking replication will tend to cause replication errors and thus mutation. Many of these mutations cause DNA repair to be less effective than normal cells and they transfer to daughter-cells by asexual reproduction, and thus resulting in cancer.² Thus, detection of DNA damage is significant for the evaluation of genotoxicity of new compounds in the early stages of development.

Many biological methods including the comet assay, unscheduled DNA synthesis test, and so on, have been used to

detect DNA damage. However, these methods are complex, time consuming, and have low efficiency and sensitivity. Developing a simple, rapid, sensitive and effective DNA detection method that can be operated by the public with accessible resources especially without suitable cell lines is one of the great challenges in analytical chemistry.³ High performance liquid chromatography,⁴ ultraviolet,^{5,6} fluorescence,^{7–9} electrochemistry,^{6,10–12} photoelectrochemistry¹³ and ECL had been used to detect DNA. Among them, nanoprobe played an important role in all these methods. With the development of nanobiotechnology, quantum dots (QDs) have attracted tremendous interest due to their unique optical, electronic, and electrochemical characteristics.^{14,15} Unfortunately, the QDs' cytotoxicity caused by the release of heavy metal ions and the instability in harsh environments limit their practical applications.¹⁶ In order to resolve these problems, a series of QD encapsulated SiO₂ nanoparticles have been prepared including CdTe@SiO₂,^{17,18} CdSe/Cd_xZn_(1–x)S@SiO₂,¹⁹ CdSe/ZnS@SiO₂,²⁰ ZnO@SiO₂ (ref. 21), etc. The silica shell not only protects the QDs from the adverse factors but also enables further surface functionalization, and has good biocompatibility, which leads to QD@SiO₂ being widely used in bioassays. When AFP was used as a model analyte, the fluorescent test strips were at least 10 times more sensitive than conventional gold-based test strips.²² A FRET system was fabricated by using ca. 200 CdTe QD encapsulated SiNPs (on average 40 nm in diameter) as ECL quenching labels and CdS:Mn nanocrystals as excellent ECL emitters to detect thrombin in the range of 5.0 aM to 5.0 fM with a detection limit of 1 aM.²³ The FRET system was

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Scheme 1 CdTe@SiO₂ was used as nanoprobe for sensitive detection of DNA damage.

also constructed for DNA detection by combining the CdSe@SiO₂ and molecular beacon, which was capable of rapid detection of the target DNA at 0.1 nM concentration within 15 min.²⁴

In this paper, CdTe@SiO₂ was synthesized by a reverse microemulsion method.^{18,25} The prepared CdTe@SiO₂ not only retained high fluorescence intensity but also avoided cytotoxicity due to the protection by the SiO₂ shell, which has been modified on the probe DNA for signal amplification. Capture DNA (C_{DNA}) was first assembled on the surface of glass carbon electrodes. DNA detection was realized by outputting a remarkable ECL signal of the CdTe@SiO₂ labeled probe DNA. When the target DNA was introduced into the system, it was complementary to the probe DNA at the one-half-segment and complementary to the capture DNA at the other half-segment, resulting in the formation of a stable duplex complex. As a result, the CdTe@SiO₂ labeled probe DNA was proximate to the surface of the electrode and the ECL was observed. When target DNA was damaged, the probe DNA cannot be introduced efficiently to the surface of the electrode and the ECL response signal decreased greatly (shown in Scheme 1). This DNA biosensor was proved to have a low detection limit and a wide dynamic range. Most importantly, the sensing system could differentiate the complementary sequence between single base mismatch sequences. The method was successfully applied to study the damage to DNA caused by several genotoxicity chemicals, which was rapid, simple, reliable and sensitive compared to the classical biological methods.

2 Experimental

2.1 Reagents and materials

All of the DNA probes were synthesized and modified by Shanghai Sangon Biotechnology Co., Ltd. (Shanghai, China) and were purified by HPLC. The sequences of all DNA used in the experiment are listed in Table 1.

Tellurium powder, cadmium chloride (CdCl₂·2.5H₂O, ≥99%), sodium borohydride (NaBH₄, 99%), and NaOH (≥99.7%) were obtained from Sinopharm Chemical Reagent Co., Ltd. (Nanjing, China). Cyclohexane (≥99.5%) and Triton

Table 1 Sequences of used DNA^a

DNA	Sequences
Capture DNA	NH ₂ -C6-AAAAAAAAAATATATTTGGTTTTCCCAA
Probe DNA	GTCCTAGGTATTCATCGCCGAAAAAAA-C7-NH ₂
Target DNA	CGGCGATGAATACCTAGGACACTTACTATTAACT GGTTTGGGAAAACCAAATATATT
Single base mismatch DNA1 (smDNA1)	CGGCGATGAA <u>AC</u> CTAGGACACTTACTATTAACT GGTTTGGGAAAACCAAATATATT
Single base mismatch DNA2 (smDNA2)	CGGCGATGAATACCTAGGACACTTACTATTAACT GGTTTGGGAAAAGCAAATATATT
Triple bases mismatch DNA1 (tmDNA1)	CGGCGATGATATCCTAGGACACTTACTATTAACT GGTTTGGGAAAACCAAATATATT
Triple bases mismatch DNA2 (tmDNA2)	CGGCGATGAATACCTAGGACACTTACTATTAACT GGTTTGGGAAAAGGTAATATATT

^a The location of single base or triple bases mismatch sites was underlined.

X-100 were purchased from Shanghai Shenbo Chemical Co., Ltd. (Shanghai, China). *n*-Hexane, ammonium hydroxide (25.0–28.0%), acetone (C₃H₆O, ≥99.5%) and ethanol were obtained from Shanghai Lingfeng Chemical Reagent Co., Ltd. (Shanghai, China). Mercaptopropionic acid (MPA, 99%) was obtained from Alfa Aesar (Tianjin, China). Tetraethoxysilane (TEOS), 3-aminopropyltriethoxysilane (APTES), bovine serum albumin (BSA), glutaraldehyde (25% in water), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC), and *N*-hydroxysuccinimide (NHS) were purchased from Sigma-Aldrich Inc. (USA). *o*-Aminobenzoic acid was donated by Yancheng Xinsheng Fine Chemistry Co., Ltd. (Yancheng, China).

All other chemical reagents were of analytical grade and were used without further purification. Phosphate buffer solution (0.1 M, pH 7.4) was prepared by mixing NaH₂PO₄·2H₂O and Na₂HPO₄·12H₂O. The stock solutions of all DNA were achieved by dissolving DNA to 100 mM with 0.1 M pH 7.4 PBS containing 0.1 M NaCl, 5 mM MgCl₂ and 5 mM EDTA.

Safety note: NaAsO₂ and other damage reagents were suspected human carcinogens and somewhat volatile. Gloves were worn, and all weighings and manipulations were done under a closed hood. All reactions were done in closed vessels.

2.2 Apparatus

A FluoroMax-4 spectrofluorometer (Horiba, Japan) was used in this experiment. UV-vis absorption spectra were recorded on a 2450 UV-visible spectrophotometer (Shimadzu, Japan). The TEM images of nanoparticles were obtained on a transmission electron microscope (JEM2100, Japan). The electrochemical impedance spectroscopy was measured on the Versa STAT 3 (Riceton Applied Research, UK). All electrochemical experiments were conducted with a CHI 830 electrochemical workstation (Shanghai Chenhua Instrument Co., Ltd., China). The ECL measurements were carried out on a MPI-E multifunctional electrochemical and electrochemiluminescent analytical

system (Xi'an Remex Analytical Instrument Co., Ltd., China). A three-electrode-system was used in all ECL experiments with a 5 mL glass cell consisting of a modified GCE working electrode, a platinum counter electrode, and an Ag/AgCl (saturated KCl solution) reference electrode.

2.3 Synthesis of CdTe QDs

The CdTe QDs were synthesized according to the previous reports.^{26,27} Briefly, sodium hydrogen telluride (NaHTe) was freshly prepared by dissolving 0.0673 g of NaBH₄ in 4 mL of nitrogen purged water and adding 0.0957 g of tellurium powder immediately. This reaction was conducted for 2 h in a 10 mL Eppendorf tube with a needle inserted for releasing the gas generated during the reaction. 0.1142 g of CdCl₂·2.5H₂O and 75 μL of MPA were added into 50 mL of nitrogen purged deionized water and sodium hydroxide solution was added dropwise to adjust the pH of the mixing solution to 9.0. Then, 2 mL of freshly prepared NaHTe solution was added into the above solution immediately under stirring. The reaction mixture was refluxed at a temperature of 100 °C for 3 h. The CdTe QDs obtained were purified by serial centrifugation and washed with water and ethanol. Finally, the resulting precipitate was dried using a thermostatic vacuum drier.

2.4 Synthesis of CdTe@SiO₂ nanoparticles

CdTe@SiO₂ core-shell structured fluorescent spheres were synthesised by a water-in-oil (W/O) microemulsion method according to Gao's description,¹⁷ whereby more uniform spheres in the size range of 30–150 nm could be obtained compared to the Stöber method. Then 7.5 mL of cyclohexane, 1.77 mL of Triton X-100, 1.8 mL of *n*-hexanol, and 100 μL of aqueous solution of as-prepared CdTe NCs (1 mg mL⁻¹) were added to a flask under stirring. The micro-scaled water pools containing CdTe QDs were stabilized by Triton X-100 and *n*-hexanol. 100 μL of TEOS and APTS, respectively, were added to the above mixing solution after it was stable for 30 min. Thus, the ethoxyl group in TEOS was replaced by trimethylamine of APTS, which decreased the electrostatic repulsion between the silica intermediate and the CdTe QDs. As a result, the number of CdTe QDs in each SiO₂ sphere increased significantly.²⁸ Subsequently, 200 μL of ammonia aqueous solution (25 wt%) was introduced to trigger the above reaction at 0 °C, which was performed in the dark under continuous stirring for 24 h. The system was kept stirring. The SiO₂ shell was formed at the oil-water interface due to the hydrolyzation of TEOS. Then, 2 mL of acetone was used to terminate the reaction. The resulting precipitates of amino-functionalized CdTe@SiO₂ composite particles were purified by repeated washing/separation with ethanol and distilled water, and were dispersed in aqueous finally. The as-prepared amino-functionalized CdTe@SiO₂ nanoparticles were reacted with glutaraldehyde solution (5 mL, 5%) at 37 °C for 2 h under stirring. The resulting solution was centrifuged and washed thoroughly with water to remove excess glutaraldehyde. The precipitates were redispersed with distilled water to a final volume of 10 mL.

2.5 Fabrication of the DNA modified glassy carbon electrode

Firstly, a clean GCE (3 mm diameter) was dipped into 1 M H₂SO₄ solution containing 50 mM *o*-ABA and scanned in a potential range of 0–1.0 V for 20 cycles at a scan rate of 40 mV s⁻¹ to an electropolymerized poly(*o*-ABA) (PAB) film. This PAB-modified electrode (PAB/GCE) was immersed in double distilled water for 30 min. Secondly, PAB/GCE was immersed in a mixture solution containing 0.1 M EDC, 5 mM NHS and 0.1 M pH 7.4 PBS for 30 min in order to activate the carboxyl group modified electrode. 8 μL of 2 μM capture DNA was dropped on the surface of this activated modified GCE and incubated for 2 hours at room temperature. It should be noted that the electrode was sealed by tubes with triangle bottom to reduce the volatilization of the organic reagent in modification solution. Then, the capture DNA-modified electrode (C_{DNA}/PAB/GCE) was soaked in 1% BSA solution at room temperature for 30 min to block the nonspecific binding sites. Thirdly, 8 μL of mixture solution containing 2 μM probe DNA and target DNA, respectively, was dropped on the surface of the C_{DNA}/PAB/GCE for 2 h incubation. Finally, 8 μL of CdTe@SiO₂ nanoprobe solution was dropped on the electrode surface for incubation for another 2 h at room temperature. It should be noted that the electrode was washed thoroughly with double distilled water and dried with nitrogen after each modification. As a result, a CdTe@SiO₂/T_{DNA}-P_{DNA}/C_{DNA}/PAB/GCE modified electrode was obtained.

2.6 ECL detection

0.1 M PBS containing 0.1 M KCl and 0.1 M K₂S₂O₈ was used as the electrolyte in a 5 mL glass cell for the ECL detection. The potential range applied to the working electrode in the CV measurement was from 0 to -1.6 V at 100 mV s⁻¹.

3 Results and discussion

3.1 Characterization of CdTe QDs and CdTe@SiO₂ nanoparticles

The UV (Fig. 1a) and fluorescence (Fig. 1b) spectrum showed that the prepared CdTe QDs had a maximum absorption peak at 620 nm and they emitted strong fluorescence at 683 nm. Fig. 1c shows that the fluorescence intensity of the CdTe@SiO₂ nanoprobes retained their strong fluorescence although it decreased when compared with CdTe QDs. Photographs of aqueous dispersed CdTe QDs (left) and CdTe@SiO₂ (right) under UV light are shown in Fig. 2A. Both of them emitted red light. The transmission electron microscopy (TEM) images showed that both CdTe QDs (Fig. 2B) and CdTe@SiO₂ nanoprobes (130 ± 10 nm in diameter, Fig. 2C and D) were monodispersed.

3.2 Electrochemical characterization of the modified electrode

The modification of the electrode was characterized by an electrochemical impedance technique (EIS). As shown in Fig. 3, the charge transfer resistance (*R*_{ct}) value corresponds to the diameter of the semicircle. At lower frequencies the linear section was due to the diffusion limited process, while at higher

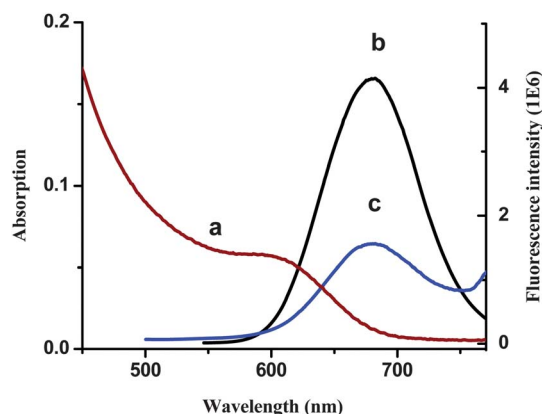


Fig. 1 UV-vis absorption spectra (a) of the CdTe QDs. Fluorescence spectra of CdTe QDs (b) and CdTe @SiO₂ nanoprobe (c).

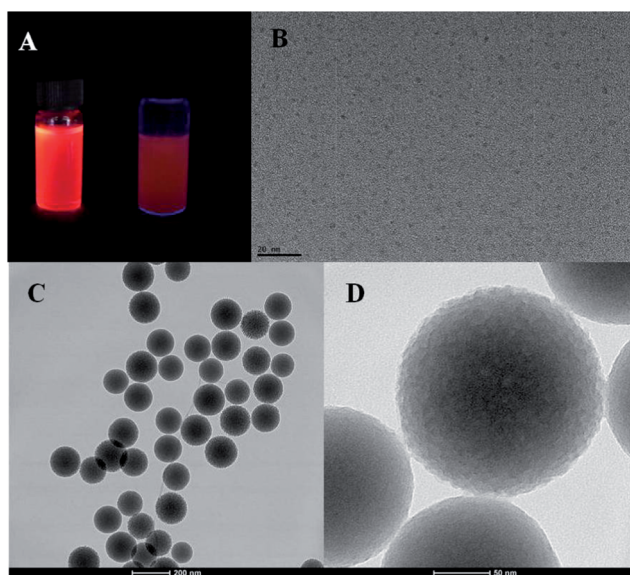


Fig. 2 Photographs of aqueous dispersion of CdTe QDs (left) and CdTe@SiO₂ nanoprobe (right) under UV light (A); TEM images of CdTe QDs (B) and CdTe@SiO₂ (C and D).

frequencies the semicircle was attributed to the electron-transfer limited process. The charge transfer process was strongly influenced by the modification of the electrode. The equivalent circuit $R_1(Q_1(R_2W))(Q_2R_3)$ was used to obtain the analog the experimental data. R_1 , R_2 , and R_3 stood for the resistance of the solution, electrolyte resistance and charge transfer respectively. W represented the Warburg resistance due to the contribution of diffusion. Q_1 and Q_2 represented the constant phase elements which were associated with the capacitance of the double layer. The electron transfer process of $[\text{Fe}(\text{CN})_6]^{3-/4-}$ at the bare electrode had a weak resistive force, so the bare GCE showed a very small semicircle domain (a). The PAB film blocked the electron transfer and resulted in the increased R_{ct} (b). The activated PAB/GCE by EDC/NHS effectively reduced the negative charge on the electrode surface and the R_{ct} decreased (c). The assembly of negatively charged capture DNA

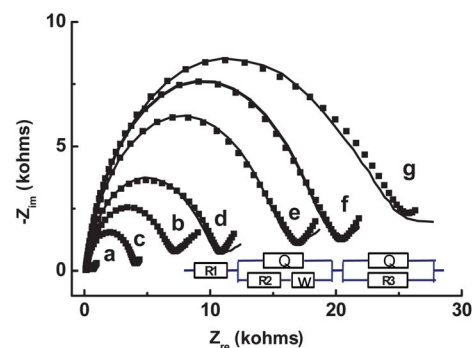


Fig. 3 Nyquist plots (line) and EIS (scatter) for (a) bare GCE; (b) PAB/GCE; (c) EDC/NHS activated PAB/GCE; (d) C_{DNA}/PAB/GCE; (e) BSA/C_{DNA}/PAB/GCE; (f) T_{DNA}-P_{DNA}/BSA/C_{DNA}/PAB/GCE; and (g) CdTe@SiO₂/T_{DNA}-P_{DNA}/BSA/C_{DNA}/PAB/GCE. The symbols represent the experimental data, while solid lines were fitted curves using equivalent circuits as shown in the inset.

repelled the electron transfer process of $[\text{Fe}(\text{CN})_6]^{3-/4-}$ at the electrode surface, the R_{ct} of the capture DNA modified electrode increased to $2727.0 \Omega \text{ cm}^{-2}$, which demonstrated that the capture DNA has self-assembled successfully on the electrode (d). Similarly, the assembly of BSA on the C_{DNA}/GCE led to a significant increase of the R_{ct} value to $3045.0 \Omega \text{ cm}^{-2}$ (e). After hybridization with the complementary target DNA and probe DNA, the R_{ct} increased to $5352.0 \Omega \text{ cm}^{-2}$ because their negatively charged phosphate backbone repelled the $[\text{Fe}(\text{CN})_6]^{3-/4-}$ anions from the electrode surface (f). The introduction of CdTe@SiO₂ nanoprobe increased the R_{ct} to $8051.0 \Omega \text{ cm}^{-2}$ (g). These results obtained from the Nyquist plots in the whole impedimetric experiment demonstrated that the sensor was successfully fabricated according to the procedures described in Section 2.5.

3.3 ECL detection of target DNA

Fig. 4A reveals the feasibility of this biosensor to be used in the detection of target DNA by using CdTe@SiO₂ nanoprobe for signal amplification. The GCE, PAB/GCE, C_{DNA}/PAB/GCE, BSA/C_{DNA}/PAB/GCE and T_{DNA}-P_{DNA}/BSA/C_{DNA}/PAB/GCE showed low ECL response (a–e) at around 750, which were the background signals. The ECL for the electrode that modified with CdTe@SiO₂ labeled DNA increased to around 9890 (f), which was

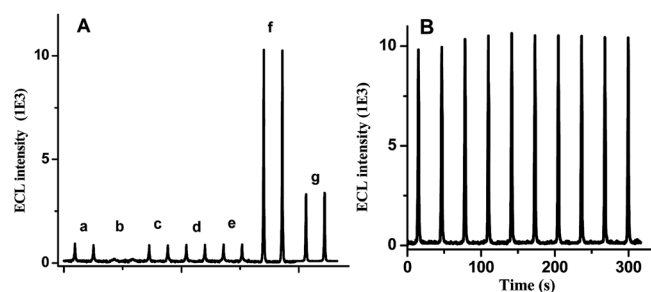


Fig. 4 (A) ECL intensity of the (a) bare GCE; (b) PAB/GCE; (c) C_{DNA}/PAB/GCE; (d) BSA/C_{DNA}/PAB/GCE; (e) T_{DNA}-P_{DNA}/BSA/C_{DNA}/PAB/GCE; (f) CdTe@SiO₂/T_{DNA}-P_{DNA}/BSA/C_{DNA}/PAB/GCE; and (g) QDs/T_{DNA}-P_{DNA}/BSA/C_{DNA}/PAB/GCE. (B) The stability of the biosensor.

12 times of that of the background signals. On the other hand, it was also about 2.5 times of that for the CdTe QDs labeled DNA modified electrode, indicating that CdTe@SiO₂ nanoprobe can amplify the signals effectively when compared to CdTe QDs. Fig. 4B shows the stable ECL intensity of this biosensor under continuous scanning for 10 cycles, which suggested that the biosensor was suitable for ECL detection.

A series of different concentrations of the target DNA were detected. As shown in Fig. 5, the ECL intensity increased with the increasing concentration of complementary T_{DNA} from 0.1 nM to 2 μM. The linear regression equation was $y = 1.011 + 0.007x$, with the regression coefficient $R = 0.9924$, where y is the ECL intensity and x is the T_{DNA} concentration. The linear response range of the sensor for the target DNA was from 0.1 nM to 2 μM with a detection limit of 0.03 nM (3σ , σ means standard deviation of the blank solution, $n = 7$).

3.4 Selectivity of the method

In order to prove the selectivity of the method, single base mismatch DNA (smDNA) and triple bases mismatch DNA (tmDNA) were detected. The concentrations of both of them were 2 μM. From Fig. 6, it could be seen that the ECL intensities for both smDNA (b and c) and tmDNA (d and e) were decreased abruptly compared to that for complementary target DNA (a). The reason was that mismatch DNA could not hybridize efficiently with C_{DNA} to form a stable duplex complex, as a result, little CdTe@SiO₂ labeled probe DNA was proximate to the electrode surface. So this biosensor was proved to be excellently selective to the specific sequence of DNA. On the other hand, it was also concluded that the location of mismatch base at the probe DNA (b and d) or the capture DNA (c and e) had little impact on the resultant ECL intensity.

3.5 Detection of DNA damage

NaAsO₂ was a toxic reagent and its damage to DNA was proved.^{29,30} Here, the constructed DNA biosensor was first used to detect DNA damage induced by it. After incubation with T_{DNA} for different times, NaAsO₂ was separated by Millipore's Amicon Ultra-0.5 centrifugal filter devices at $14\,000 \times g$. Then, these treated DNA was diluted to its original concentration and

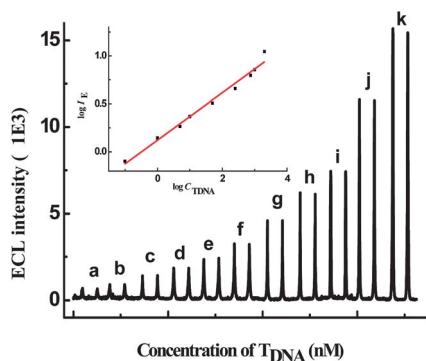


Fig. 5 Influence of different concentrations of T_{DNA} on the ECL intensity. 5 μM C_{DNA}; 0, 0.1, 1, 5, 10, 50, 250, 750, 1000, 2000, 5000 nM T_{DNA} from curve a to k. Inset: calibration curve for detection of T_{DNA}.

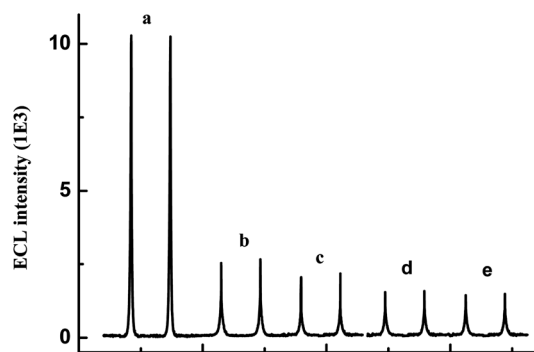


Fig. 6 ECL intensity in the presence of complementary T_{DNA} (a), smDNA1 (b), smDNA2 (c), tmDNA1 (d), and tmDNA2 (e). DNA concentration was 2 μM.

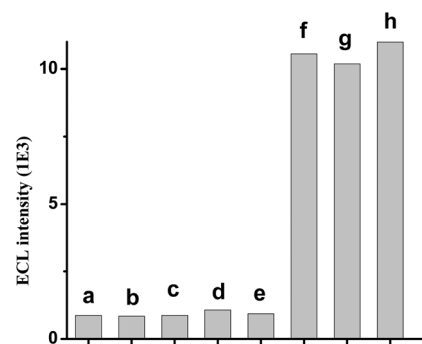


Fig. 7 ECL intensity of the DNA biosensor in the presence of 2 μM T_{DNA} treated by (a) NaAsO₂; (b) styrene; (c) SO; (d) 3 ng per mL AFT; (e) 3 ng per mL ochratoxin; (f) methanol (25% (v/v)); (g) ethanol (25% (v/v)) and (h) intact T_{DNA}. The concentrations of C_{DNA} and R_{DNA} were 2 μM, respectively.

detected according to the procedure described in Section 3.3. The biosensor showed high ECL signals in the presence of intact target DNA, while its intensity decreased nearly to the background signals in the presence of target DNA that treated with NaAsO₂ for more than 45 minutes. The damage to the target DNA by NaAsO₂ has been proved.

DNA damage from other chemicals such as styrene, epoxy-styrene (SO), aflatoxins (AFT), ochratoxin, methanol and ethanol were also studied by the biosensor. Fig. 7 shows that, similarly to NaAsO₂, ECL intensities in the presence of styrene, SO, AFT and ochratoxin treated target DNA decreased nearly to the background signals (a–e). These results indicated that all these chemicals induced damage to DNA, which were in agreement with the reported results.^{2,31,32} It also concluded that solvents such as methanol (f) and ethanol (g)³³ have little damage to DNA in 2 h because the ECL signals of this biosensor changed slightly in comparison with the intact DNA.

4 Conclusion

In this work, an ECL biosensor was fabricated to detect specific sequences of DNA by using CdTe@SiO₂ as nanoprobe for signal amplification. It had a low detection limit of 0.03 nM and a wide dynamic range from 0.1 nM to 2 μM. In addition, the method had good specificity to differentiate the complementary

sequence between single base mismatched sequences. The method has been successfully applied to detect DNA damage induced by chemical reagents such as SO, NaAsO₂, AFT, and ochratoxin. Solvents such as methanol and ethanol at dosage levels as used in the present experiment cause little damage to DNA. In summary, this method for detection of DNA damage was simple, reliable and sensitive.

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