Electrochemiluminescence immunosensor for highly sensitive detection of 8-hydroxy-2'-deoxyguanosine based on carbon quantum dot coated Au/SiO₂ core-shell nanoparticles

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Abstract

An electrochemiluminescence (ECL) immunosensor using Pt electrode modified with carbon quantum dot (CQDs) coated Au/SiO₂ core-shell nanoparticles was proposed for sensitive detection of 8-hydroxy-2'-deoxyguanosine (8-OHdG) in this work. Rabbit anti-8-OHdG antibody was covalently bound to CQDs on the surface of Au/SiO₂ core-shell nanoparticles. Through signal amplification of Au/SiO₂ core-shell nanoparticles, 8-fold enhancement of the ECL signals was achieved. Under optimal conditions, a good linear range from 0.2 to 200 ng mL⁻¹ with a low detection limit of 0.085 ng mL⁻¹ (S/N = 3) for 8-OHdG detection was obtained. Interfering substances tests showed that the corresponding ECL intensity (Δ ECL) of 8-OHdG is 8 to 18 times higher than that of guanine, uric acid (UA) and ascorbic acid, demonstrating its good selectivity for 8-OHdG detection. The ECL immnuosensor exhibits long-term stability with a relative standard deviation (RSD) of 8.5% even after 16 cycles of continuous potential scans. The result of analytical detection of 8-OHdG in real samples was satisfactory. The proposed ECL immnuosensor shows good performance with high sensitivity, specificity, repeatability, stability and provided a powerful tool for 8-OHdG monitoring in clinical samples.

Keyword: Core-shell nanoparticle , 8-hydroxy-2'-deoxyguanosine ,

Electrochemiluminescence immnuosensor , Carbon quantum dot

1. Introduction

Reactive oxygen species, such as superoxide radical, singlet oxygen, hydroxyl radicals, superoxide anion and peroxy, are considered as harmful species due to their effect on oxidative DNA damage [1,2]. 8-hydroxy-2'-deoxyguanosine (8-OHdG, also known as 8-oxo-7, 8-dihydroguanine and 8-oxo-dGuo) (Scheme 1), one of the main products of oxidative DNA damage, can mispair with adenine, leading to G:C→T:A transverse mutation [3]. Many researches show that 8-OHdG has a much closer relationship with some disease, such as cancer, diabetes and neurological disorders [4-6]. Therefore, 8-OHdG is concerned as the most investigated biomarker of oxidative DNA damage [7].

Traditional methods for 8-OHdG determination, such as high performance liquid chromatography with electrochemical detection [8], liquid chromatography combined with electrospray ionization tandem mass spectrometry multiple reaction monitoring [9], high performance liquid chromatography/positive electrospray ionization tandem mass spectrometry [10], enzyme-linked immuno sorbent assay [11] and ³²P-labeling [12], are pretreatment complex, specialized skills required and unavailable for in-situ detection. Electrochemistry sensors are widely applied in detecting various pollutants due to its rapid and high sensitivity [13,14]. It also been developed for 8-OHdG detection. For example, electrochemical method based on poly(3-methylthiophene) (P3MT) and polyethylenimine dispersed carbon nanotubes modified glassy carbon electrode (GCE) used as working electrode allowed the sensitive determination of 8-OHdG. But the results of electrochemistry methods above were likely to be affected by other biomolecules or matrix effects [15,16]. Fluorescent sensor is another method for 8-OHdG detection which developed in recent years. Qian Zhang et al. [2] reported a DNA aptamer fluorescent sensor for 8-OHdG determination using two triple-stranded DNAs as the scaffolds, which shows inherently insufficient binding constants between aptamers and 8-OHdG due to the small molecule of 8-OHdG.

Electrochemiluminescence (ECL) is a special kind of chemiluminescence (CL) with its light emission preceded by electrochemical reactions [17]. The ECL method presents widespread prospect due to its high sensitivity and good controllability

compared to the conventional electrochemical and CL technique [18]. Its performance mainly depends on the favourable luminescent characterization of ECL materials. Recently, as a new group of ECL luminophores, quantum dots (QDs) attracted more and more attention [19-21], since they not only exhibit excellent ECL activities but also have the merit of easy labeling. However, reports on QD-based ECL assay for 8-OHdG detection are relatively scarce since the inherently toxicity and low ECL intensity due to the poor stability of conventional QDs used in ECL at high electrochemical potentials [22]. Therefore, nontoxicity and reproducibility are urgently needed for the development of QD-based ECL sensors in 8-OHdG monitoring.

Carbon quantum dots (CQDs), a newly emerging ECL luminophores, have several significant advantages over the previously reported QDs, such as high stability, low toxicity, easy labeling and good biocompatibility [23-25]. To the best of our knowledge, the research report of CQD-based ECL 8-OHdG is hampered by the low ECL intensity of CQDs and the difficulty in reuse of CQDs in an aqueous system [26,27]. Thus, how to effectively amplify ECL signal and solve the reusability simultaneously is the key point in designing a CQDs-based ECL for 8-OHdG detection.

A structure of “core-shell” based on concentric multilayer semiconductor nanoparticles has recently attracted great attention in sensor field due to its large surface-to-volume ratio for modifying QDs. Nanguo Liu et al. [28] reported a well-defined hybrid structures that comprised a gold core coated with a silica shell (Au/SiO₂), followed by the modification of CdSe-QDs (Au/SiO₂/CdSe-QD). The research on the photoluminescence intensities of CdSe-QD and the Au/SiO₂/CdSe-QD nanoparticles prove that the Au/SiO₂ core-shell nanoparticles exhibit excellent ability of signal amplification on photoluminescence. Moreover, due to its robust chemical stability, uniformity and biocompatibility of the SiO₂ shell coating, and ease of surface functionalization, we consider Au/SiO₂ core-shell can be used as suitable platform for CQDs immobilization to improve its ECL signal and

reusability.

Herein, an advanced ECL immunosensor based on the hybrid of CQDs and Au/SiO₂ was fabricated for the supersensitive, rapid, and selective detection of 8-OHdG upon the amplification of CQD ECL signal by Au/SiO₂ core-shell. In the sensor Au/SiO₂ core-shell nanoparticles serve as the platform for CQDs immobilization, which can not only enhance the detection sensitivity but also achieve good recycle of the sensor through avoiding the loss of CQDs. Meanwhile the rabbit anti-8-OHdG antibody serves as the immunosorbent of antigen 8-OHdG. Compared to the previously reported methods, this approach provides a promising tool for the routine detection of 8-OHdG.

2. Experimental

2.1 Reagents and chemicals

8-OHdG (CAS: 88847-89-6), ethyl-3-(dimethyl aminopropyl) carbodiimide (EDC) and N-hydroxysuccinimide (NHS) were purchased from Sigma (America). Rabbit anti-8-OHdG (Ab) was obtained from Beijing Biosynthesis Biotechnology Co., Ltd. (Beijing, China). HAuCl₄•3H₂O (99%), tetraethoxysilane (TEOS), poly(vinylpyrrolidone) (PVP) and trisodium citrate (99%) were bought from Xilong Chemical Co., Ltd. (Guangdong, China). NH₄OH was purchased from Tianjin Fuyu Fine Chemical Co., Ltd. (Tianjin, China). Uric acid (UA) was purchased from Tokyo Chemical Industry Co., Ltd. (Japan). Guanine and (3-Aminopropyl) trimethoxysilane (APS) were supplied by J&K Scientific Ltd. (Beijing, China). Bovine serum albumin (BSA) was purchased from Beijing Aoboxing Bio-tech Co., Ltd. (Beijing, China). Ascorbic acid, K₂S₂O₈ (potassium persulfate) and KCl were bought from Tianjin Bodi Chemical Co., Ltd. (Tianjin, China). Phosphate buffer solution (PBS, 0.1mol L⁻¹, pH=7.4) was prepared by mixing the stock solutions of Na₂HPO₄ (0.1 mol L⁻¹) and NaH₂PO₄ (0.1 mol L⁻¹). Tris-EDTA (TE) buffer was used as dilution solvent of 8-OHdG. Ultrapure water purified by a Millipore water system (resistivity > 18.0 MΩ cm⁻¹, Laikie Instrument Co., Ltd., Shanghai, China) was used throughout the experiments.

2.2 Apparatus

The ECL emission was detected by a MPI-B multi-parameter chemiluminescence analysis test system (Xi'an Remax Analysis Instrument Co., Ltd., Xi'an, China) with a three electrode system consisting of a bare or modified Pt electrode (1 mm-diameter) as the working electrode, the standard calomel electrode (SCE, saturated KCl) and a Pt wire were used as the reference electrode and the auxiliary electrode, respectively. Electrochemical impedance spectroscopy (EIS) was performed on a CHI 660 electrochemical analyzer (Shanghai Chenhua Instrument, Shanghai, China). Unless noted, the photomultiplier tube (PMT) was 600 V. The morphology of the samples was observed by transmission electron microscopy (TEM) (FEI Tecnai G2 20, America) and scanning electron microscopy (SEM) (Hitachi S-4800) equipped with an X-ray energy dispersive spectroscopy (EDS) (Japan). Photoluminescence (PL) spectra were obtained on a Hitachi F-4500 fluorescence spectrometer (Japan). UV-visible spectra were carried out on a Jasco V-550 spectrometer (Japan). Sonications were carried out on a KQ5200B-type ultrasonic cleaner with the cleaning slot size 300*240*150 mm (Kun Shan Ultrasonic Instruments Co., Ltd., Jiangsu, China).

2.3 Synthesis of Au/SiO₂/CQDs core-shell nanoparticles

Preparation of CQDs: Ascorbic acid was used as carbon source of CQDs. The mixed liquor of ascorbic acid and ethanol was heated at 180 °C for 4 h in a Teflon®-lined stainless steel autoclave and then cooled to room temperature. The dark brown solution was obtained, followed by extracting with dichloromethane. Then a yellow aqueous solution containing CQDs was obtained. The specific processes refer to previous literature [29].

Preparation of Au nanoparticles (AuNPs): The AuNPs core was synthesized through the seeded growth synthesis process according to literature [30]. Briefly, 2.2 mmol L⁻¹ sodium citrate solution (150 mL) was added into a 250 mL round-bottomed flask. The solution was heated reflux under vigorous stirring. When the solution start boiling, 1 mL of HAuCl₄ (25 mmol L⁻¹) was injected and kept boiling for 10 minutes.

Then cooled the mixtures to 90 °C. 1 mL of sodium citrate (60 mmol L⁻¹) and 1 mL of HAuCl₄ solution (25 mmol L⁻¹) were sequentially injected, then kept reacting for 30 min at 90 °C. After repeating this process steps (sequential addition of 25 mmol L⁻¹ HAuCl₄ (1 mL) and 60 mmol L⁻¹ sodium citrate (1 mL) 5 times, a vinicolor aqueous solution containing AuNPs was finally obtained.

Preparation of Au/SiO₂ core-shell nanoparticles: The Au/SiO₂ core-shell nanoparticles were synthesized by Stöber process in accordance to literature [31]. An aqueous solution of poly(vinylpyrrolidone) (PVP, 1 mL, 12.6 mgmL⁻¹) was added to the 180 mL aqueous solution of as-prepared AuNPs. Subsequently, the mixture stood for 48 h under magnetic stirring at room temperature, assuring the attachment of PVP molecules on the surfaces of AuNPs. The PVP attached AuNPs were then separated from solution by centrifuging at 12,000 rpm for 30 min and re-dispersed in 15 mL of ultrapure water. Thereafter, transfer 5.0 mL of PVP-modified AuNPs into a beaker containing a total volume of 15 mL mixture of ammonia (0.4 mL) and ethanol (14.6 mL) under magnetic stirring. The mixture composed of TEOS (0.1 mL) and ethanol (9.9 mL) were injected into the beaker within a period of 6 h. Then stirring reaction was kept for 12 hours. The synthesized Au/SiO₂ core-shell nanoparticles were washed and centrifuged for two times by ethanol and ultrapure water in turn.

Formation of Au/SiO₂/CQDs core-shell nanoparticles: The synthesis process (shown in Scheme 2) was carried out according to previous literature [28]. The as-prepared Au/SiO₂ nanoparticles were dispersed in ethanol (20.0 mL). 2 μL of APS was added to the solution to modify the silica surface with amine groups. After that the mixture was magnetic stirred for 24 h at room temperature. The amine-functionalized Au/SiO₂ core-shell nanoparticles were collected by centrifugation and washed twice with ethanol. The as-prepared nanoparticles were re-dispersed in 20.0 mL of ethanol. Before immobilized on the amine-functionalized Au/SiO₂ nanoparticles, the hydroxyl groups on the surface of CQDs were converting into carboxyl by adding 0.005 g of NaOH and 0.01 g of ClCH₂COOH (chloroacetic acid) into 2 mL of CQDs solution and bath sonicating for 3 h (frequency: 40 KHZ,

power: 200 W) [32]. Then the mixture was added into ethanol solution of amine-functionalized Au/SiO₂ core-shell nanoparticles, and kept magnetic stirring for 12 h at room temperature. CQDs were self-assembled onto Au/SiO₂ nanoparticle surface due to the strong binding interaction between the -NH₂ and the -COOH of the CQDs. Finally, the hybrid Au/SiO₂/CQDs nanoparticles were collected by centrifugation and washed twice with ethanol and ultrapure water in turn.

2.4 Preparation of the ECL immunosensor

Prior to modification, the Pt electrode (1 mm-diameter) was mildly polished with 0.5 μm and 50 nm alumina slurry and then washed ultrasonically in ultrapure water and ethanol for a few minutes, respectively. 2 μL of the Au/SiO₂/CQDs nanoparticles suspension was dropped on the pretreated Pt electrode (noted as Pt/Au/SiO₂/CQDs) and then naturally dried at room temperature. The Pt/Au/SiO₂/CQDs electrode was carefully immersed in a solution containing 0.01 mol L⁻¹ EDC and 0.025 mol L⁻¹ NHS at least 12 h for activation. 2 μL of 10 $\mu\text{g mL}^{-1}$ Ab was dropped on the electrode surface and kept for at least 12 h at 4 °C (noted as Pt/Au/SiO₂/CQDs/Ab). Then, 2 μL of 1% BSA was dropped on the electrode surface and kept for 3 h to block the nonspecific sites (noted as Pt/Au/SiO₂/CQDs/Ab/BSA). Finally, the ECL immunosensor was obtained (the whole procedure was presented in Scheme 3). Notably, after every step, the electrode was rinsed with 0.01 mol L⁻¹ PBS (pH 7.4).

2.5 Immunoassay of 8-OHdG

The detection procedures of 8-OHdG are presented in Scheme 4. In summary, the modified electrode was immersed in the incubation solution containing various concentration of 8-OHdG for 10 h and this process was performed at 37 °C isothermally in the incubator. After that the modified electrode was rinsed with 0.01 mol L⁻¹ PBS (pH 7.4) thoroughly. The modified electrode above was scanned in 0.1 mol L⁻¹ PBS (pH 7.4) containing 0.1 mol L⁻¹ KCl and 0.05 mol L⁻¹ K₂S₂O₈. The voltage of PMT was maintained at 600 V and the potential range was from -0.2 V to -1.0 V with a scan rate of 0.01 V s⁻¹.

3. Results and discussions

3.1 Characterization of CQDs

To evaluate the photoluminescence properties of CQDs the photoluminescence spectra and UV/Vis absorption were obtained on the fluorescence spectrometer and the spectrometer (Fig. 1). The photoluminescence intensities of CQDs were obvious under the excitation with different wavelength (a: 300 nm-g: 420 nm). The photoluminescence spectra were shifted to longer wavelengths with the increase of excitation wavelength. An optical absorption was obtained in UV region. These results are almost consistent with the data reported in previous literature [33]. It indicates that the photoluminescence intensity of CQDs was satisfied for the ECL immunosensor.

3.2 Characterization of Au/SiO₂/CQDs core-shell nanoparticles

TEM was used to investigate the morphologies of Au/SiO₂/CQDs core-shell nanoparticles. Fig. 2A shows TEM image of Au/SiO₂/CQDs core-shell nanoparticles. The diameters of AuNPs were found in the range of 20 nm to 30 nm, and the silica-shell thicknesses were between 25 nm and 30 nm. The AuNPs and the Au/SiO₂/CQDs core-shell nanoparticles were nearly spherical and apparently well-dispersed. To further verify the successful synthesis of Au/SiO₂/CQDs core-shell nanoparticles, EDS was used to investigate the elementary composition of Au/SiO₂/CQDs core-shell nanoparticles. It was clearly that Au, Si, O, and C were the elementary components of Au/SiO₂/CQDs core-shell nanoparticles (Fig. 2B), and the atomic ratios of them were 15.33%, 27.79%, 25.41% and 15.33%, respectively (table 1). The EDS results from different regions were similar, which confirmed that the composition of the obtained materials were uniform.

3.3 ECL behaviors of the ECL immunosensor

The ECL behaviors of the immunosensor were recorded in 0.1 mol L⁻¹ PBS (pH 7.4) containing 0.1 mol L⁻¹ KCl and 0.05 mol L⁻¹ K₂S₂O₈, and ECL electrodes were scanned from -0.2 to -1.0 V (Fig. 3). The ECL signal of Pt electrode was very weak (curve a). After the modification with CQDs, the ECL intensity increased. It was

noted that the ECL intensity of Au/SiO₂/CQDs nanoparticles modified electrode (curve f) was about 8 times as high as that of the CQDs modified electrode (curve b), which illustrates that the Au/SiO₂ nanoparticles have an excellent ability of signal amplification. A smaller ECL intensity (curve c) than that of in curve f was observed for Pt/Au/SiO₂/CQDs/Ab, indicating that Ab has been immobilized on the surface of electrode. After the electrode was encapsulated with BSA (curve d), the successive decline of ECL intensity was detected. Mainly depending on antibody and BSA as proteins were electron inert and they could partially hinder the electron transfer on the surface of working electrode [34]. The ECL intensity further decreased after the electrode was incubated with 8-OHdG sample (100 ng mL⁻¹) (curve e). It indicates that 8-OHdG was specifically bound to antibody, in the meantime the electron transfer on the surface of working electrode was hindered by 8-OHdG. Therefore the electrode modified by Au/SiO₂/CQDs/Ab/BSA could be used for the detection of 8-OHdG.

3.4 Optimization parameters

Highly acidic or alkaline surroundings could reduce the biological activity of the protein [35] and further affect the ECL signal of ECL immunosensor. To achieve an optimal ECL signal, pH value of electrolyte solution 8-OHdG was investigated. As shown in Fig. 4A, a maximum value of the ECL intensity achieved when the pH value of electrolyte solution was adjusted to pH 7.4. Hence neutral surroundings at pH 7.4 was selected and used for test throughout this study. The incubation time was another effect factor of the ECL signal, thus different incubation times of 8-OHdG were studied in this part. Fig. 4B shows the effect of incubation time of 8-OHdG on ECL emission. The ECL emission was decreased with incubation time from 4 to 10 h, and a relatively stable value was obtained when the incubation time of 8-OHdG up to 10 h. Therefore, 10 h of the incubation time was chosen for subsequent experiments.

3.5 ECL detection of 8-OHdG

Under the optimal conditions, the linear response range and the detection limit of the 8-OHdG sensing system were measured as follows. The working electrode after

incubating with a group of 8-OHdG concentrations (0, 0.2, 0.5, 1, 5, 10, 25, 50, 100, 150 and 200 ng mL⁻¹) were investigated to evaluate the sensitivity of the ECL immunosensor. With the increase of concentration, the ECL response was reduced (Fig. 5A). The ECL intensity reduced linearly with the concentration of 8-OHdG in the range of 0.2 to 200 ng mL⁻¹ (Fig. 5B). The regression equation is $\Delta\text{ECL} = 171.688 + 249.011 x$, with a correlation coefficient (R^2) of 0.937. The limit of detection (LOD) was 0.085 ng mL⁻¹ (S/N = 3). The low LOD might be attributed to the excellent ability of signal amplification with the Au/SiO₂ core-shell nanoparticles, and it is satisfactory to detect the concentration of 8-OHdG in some human biomatrices (the concentration of 8-OHdG in human urine, seminal plasma and breast milk were 2.3-9.3, 0.444-1.047 and 0.178-0.339 ng mL⁻¹, respectively, these data were mentioned in previous literature [36]). Although the LOD of the ECL immunosensor might not satisfy the ranges of all the biological samples, it has a relatively large linear range and a low detection limit compared to previous methods (Table 2).

3.6 Specificity, reproducibility, stability and feasibility

For many 8-OHdG detection methods, false positive or errors caused by interferences which co-exist with 8-OHdG in human body or excretion inhibited the accurate detection of 8-OHdG. To examine the specificity of the fabricated immunosensor, interferences study was performed using guanine, UA, and ascorbic acid, which were co-exist with 8-OHdG. Here, the interfering substances mentioned above with three concentration levels (100 ng mL⁻¹, 1000 ng mL⁻¹ and 10000 ng mL⁻¹) were detected. By comparison the response signals of the interferences with 8-OHdG in 100 ng mL⁻¹, the ΔECL of the interferences is only 5.5% - 12.5% of that of 8-OHdG (Fig. 6). The result indicated that these interfering substances have little impact on the detection of 8-OHdG, even their concentrations were 100 times higher than that of 8-OHdG.

The reproducibility and operational stability were the major concerns for practical application of immunosensors. The reproducibility of the ECL immunosensor was investigated by prepared five electrodes for the detection 100 ng

mL^{-1} of 8-OHdG. The relative standard deviation (RSD) of this measurement was 6.8%. The experimental results demonstrated acceptable reproducibility of the ECL immunosensor. Fig. 7 displays the ECL emission of the ECL immunosensor under 16 cycles of continuous potential scans between -0.2 and -1.0 V (vs SCE) in 0.1 mol L^{-1} PBS (pH7.4) at 0.01 V s^{-1} for the detection of 100 ng mL^{-1} of 8-OHdG. A stable ECL signal was observed with the RSD of 8.5%. The life time of the immunosensor was also investigated in 30-day period. The modified electrode was stored in the refrigerator at 4°C and measured every 10 days. During the first 10 days, the response current decreased by 1.3% of its initial response. In the next 10 days and the last 10 days it decreased by 3.7% and 6.5%, respectively, and maintained more than 93.5% of the initial response after storing for 30 days. It shows that the stability of the immunosensor was excellent and the life time of the immunosensor could be reach 30 days.

To monitor the feasibility of the developed ECL immunosensor in real samples analyses, the immunosensor was further applied in analyzing the concentrations of 8-OHdG in milk. Recovery experiments were carried out by the standard addition method. The results showed that the recovery ranges from 102.0% to 110.0%, and that the corresponding RSD ranges from 4.9% to 7.7% (Table 3). Therefore, the ECL immunosensor could be satisfactorily applied to the detection of 8-OHdG in real samples.

4. Conclusions

In this work, we developed an ECL immunosensor based on Au/SiO₂/CQDs core-shell nanoparticles for detection of 8-OHdG. ECL emission signal of CQDs increased greatly by using Au/SiO₂ core-shell nanoparticles as the substrate material. With the amplification of ECL signal, the ECL immunosensor displayed a highly sensitive ECL detection with specific immunoreactions. Under optimal conditions, the proposed immunosensor for 8-OHdG exhibited a low detection limit (0.085 ng mL^{-1}) and a wide linear range ($0.2\text{-}200 \text{ ng mL}^{-1}$). Moreover, this method was successfully applied to the detection of 8-OHdG in real samples. Given the excellent selectivity of

the ECL immunosensor coupled with acceptable repeatability and stability, this ECL immunosensor could be promising for clinical detection of 8-OHdG. It is expected that to improve the application of CQDs in biological detection by synthesizing CQDs coated Au/SiO₂ core-shell nanoparticles.

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Figure captions

Scheme 1. Chemical structure of 8-OHdG.

Scheme 2. Schematic illustration of the preparation procedure of Au/SiO₂/CQDs core-shell nanoparticles.

Scheme 3. Schematic illustration of the preparation processes of the ECL electrode modified by Au/SiO₂/CQDs core-shell nanoparticles and antibody.

Scheme 4. Schematic illustration of the detection procedures of the ECL immunosensor.

Fig. 1. Photoluminescence emission spectra (the left coordinates) and UV/Vis absorption spectra (Abs) (the right coordinates) of the CQDs, excitation wavelength: (a) 300 nm, (b) 320 nm, (c) 340 nm, (d) 360 nm, (e) 380 nm, (f) 400 nm, (g) 420 nm.

Fig. 2. (A) TEM images of Au/SiO₂/CQDs core-shell nanoparticles (inset: AuNPs). (B) EDS of Au/SiO₂/CQDs core-shell nanoparticles (inset: SEM images).

Fig. 3. The ECL signals of (a) bare Pt electrode, (b) Pt/CQDs, (c) Pt/Au/SiO₂/CQDs/Ab, (d) Pt/Au/SiO₂/CQDs/Ab/BSA, (e) Pt/Au/SiO₂/CQDs/Ab/BSA/8-OHdG, (f) Pt/Au/SiO₂/CQDs in 0.1 mol L⁻¹ PBS solution (pH 7.4) containing 0.1 mol L⁻¹ KCl and 0.05 mol L⁻¹ K₂S₂O₈. Scan rate, 0.01 V s⁻¹. The voltage of PMT was 600 V.

Fig. 4. (A) Effects of pH value of PBS solution and (B) incubation time of 8-OHdG on the response of ECL immunosensor for the detection of 100 ng mL⁻¹ 8-OHdG (n = 3).

Fig. 5. (A) ECL responses of the proposed ECL immunosensor to different concentrations of 8-OHdG (0, 0.2, 0.5, 1, 5, 10, 25, 50, 100, 150 and 200 ng mL⁻¹) in 0.1 mol L⁻¹ PBS solution (pH 7.4) containing 0.1 mol L⁻¹ KCl and 0.05 mol L⁻¹ K₂S₂O₈. Scan rate, 0.01 V s⁻¹. The voltage of PMT was 600 V. (B) The calibration curve for 8-OHdG assay (n = 3).

Fig. 6. ECL responses of the proposed ECL immunosensor of 8-OHdG, uric acid, ascorbic acid, and guanine at concentration of 100 ng mL⁻¹, respectively (n=3). All measurements were performed in 0.1 mol L⁻¹ PBS solution (pH 7.4) containing 0.1

mol L⁻¹ KCl and 0.05 mol L⁻¹ K₂S₂O₈. Scan rate, 0.01 V s⁻¹. The voltage of PMT was 600 V.

Fig. 7. The ECL intensities of sixteen consecutive cycles of the proposed ECL immunosensor incubated with 100 ng mL⁻¹ 8-OHdG in 0.1 mol L⁻¹ PBS solution (pH 7.4) containing 0.1 mol L⁻¹ KCl and 0.05 mol L⁻¹ K₂S₂O₈. Scan rate, 0.01 V s⁻¹. The voltage of PMT was 600 V.

Table 1 The atomic ratios of Au/SiO₂/CQDs core-shell nanoparticles.

Element	Intensity (c/s)	Wt%
C	24.46	15.333
O	75.52	25.461
Si	281.10	27.795
Au	213.85	31.412

Table 2 Comparison between the described ECL immune method and referenced methods for 8-OHdG detection.

Detection method	Linear range (ng mL ⁻¹)	Detection limit (ng mL ⁻¹)	References
DNA aptamer sensor	0.849-283	-	[2]
HPLC-ECD	-	0.34	[8]
Electrochemical	198.1-9905 9905-19800	28.3	[13]
Electrochemical	141.5-8490	28.3	[14]
ECL immune method	0.2-200	0.085	This paper

Table 3 Determination of 8-OHdG in milk and recoveries of the samples with different concentrations of 8-OHdG.

Sample namble	Added (ng mL ⁻¹)	Measured $\pm$ SD (ng mL ⁻¹)	Recovery (%)	RSD (%, n=3)
1	0.5	0.51 $\pm$ 0.02	102.0	5.2
2	1	1.11 $\pm$ 0.08	110.0	7.6
3	10	10.68 $\pm$ 0.82	107.0	7.7
4	50	51.60 $\pm$ 2.52	103.2	4.9
5	100	103.10 $\pm$ 5.99	103.1	5.8

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6

Figure 7

scheme 2

scheme 3

A table of contents entry

A rapid, and highly sensitive electrochemiluminescence immunosensor based on carbon quantum dot coated Au/SiO₂ core-shell nanoparticles is presented for 8-hydroxy-2'-deoxyguanosine detection.