Development of microplate-based photoelectrochemical DNA biosensor array for high throughput detection of DNA damage

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ABSTRACT

Many chemicals have been found to induce DNA damages which may lead to gene mutation and tumor generation. In this report, a microplate-based photoelectrochemical DNA sensor array was developed for the rapid and high throughput screening of DNA damaging chemicals. A 96-well plate with built-in electrodes was fabricated on a plastic substrate by the standard electronics industry processes. The working electrode in each well was deposited with SnO₂ nanoparticles, and the resulting film was sintered at low temperatures tolerable for the plastic substrate. The film was characterized by scanning electron microscopy, X-ray diffraction and X-ray photoelectron spectroscopy. On the plates sintered at 150 °C, a significant amount of photocurrent was obtained in a Ru(bpy)₃²⁺ (bpy = 2,2'-bipyridine) solution. To construct a DNA sensor, poly-(diallyldimethyl ammonium chloride) and double-stranded DNA were sequentially assembled on the SnO₂ electrode by electrostatic interaction, and a DNA intercalator, Ru(bpy)₂(dppz)²⁺ (dppz = dipyrro[3,2-a:2',3'-c]phenazine) was used as the photoelectrochemical signal indicator. After the DNA film was exposed to tetrafluoro-1,4-benzoquinone (TFBQ) or TFBQ/H₂O₂, the photocurrent dropped by 38% and 73% respectively. The photocurrent reduction can be attributed to less binding of Ru(bpy)₂(dppz)²⁺ to the electrode after DNA damage. Photocurrent measurement of the entire 96-well plate was completed within 22 min automatically.

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1. Introduction

According to official statistics, currently there are around 100,000 chemicals on the world market. In addition, about 4000 new chemicals are produced each year. The sad truth is that safety data for the large majority of these chemicals are very scarce. This lack of information poses potentially very serious threat to human health and ecosystems [1]. Among the tested chemicals, 107 are carcinogenic to humans (Group 1), and 326 chemicals are probably or possibly carcinogenic to humans (Group 2) [2]. Some of these carcinogens are geno-toxic, which act directly on genes and induce DNA damages. If unrepaired, damaged DNA can lead to gene mutation, and may eventually develop into cancer.

It has been generally recognized that there are two common types of chemically induced DNA damages, DNA oxidation by reactive oxygen species (ROS), and DNA adduct formation with exogenous chemicals and their *in vivo* metabolites. Some transition metal ions such as Fe²⁺ and Cu²⁺ have been found to react with H₂O₂ to produce extremely reactive hydroxyl radicals by the so-called Fenton reaction. The radical can induce several classes of oxidative

DNA damage, including single-strand break, double-strand break, abasic sites, and base oxidation [3]. Polycyclic aromatic hydrocarbons (PAHs) are an important class of air pollutants generated from incomplete burning of organic matters such as automobile exhausts and cigarette smoking. Benzo-[a]-pyrene, one member of the PAH family with five fused benzene rings, is a confirmed carcinogen. It is converted *in vivo* by metabolizing enzymes to a reactive intermediate, benzo-[a]-pyrene diol epoxide, which is capable of forming covalent adduct with the amine groups on DNA bases [4].

To test for the genetic toxicity of an unknown chemical, it is recommended that at least three cell-based assays should be performed, including gene mutation assay in bacteria, gene mutation assay in mammalian cells, and chromosomal aberration test in mammalian cells [5]. Although these assays have relatively high sensitivity and specificity for genetic toxicity screening, the drawbacks are obvious, such as low throughput, long duration, and high complexity. There are also a few established analytical methods for the identification and quantification of DNA damage products which can be used as molecular markers for genetic toxicity [6]. 8-Oxo-guanine (8-HO-dG) is a common product of oxidative DNA damage, and can be identified using HPLC separation followed by online electrochemical detection [7]. This method is very specific, but it requires relatively large and expensive equipments. The throughput is also very low. ³²P post-labeling is the most widely

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used method for the identification and quantification of DNA adducts [8,9]. The procedure, however, is very time-consuming, including enzymatic digestion of DNA sample, purification, labeling with ^{32}P , and TLC or PAGE analysis.

To overcome the difficulties associated with the above methods and techniques, toxicity sensors have been proposed and developed as a rapid screening tool for the identification of hazardous chemicals. For instance, DNA damage was investigated with electrochemical and electrochemiluminescent sensors and sensor arrays by a number of research groups [10–15]. We also developed a photoelectrochemical DNA sensor for the rapid detection of chemical-induced DNA damage [16–19]. The sensor was fabricated by assembling a double-stranded DNA film on SnO_2 electrode surface. A DNA intercalator, $\text{Ru}(\text{bpy})_2(\text{dppz})^{2+}$, was allowed to bind to the DNA film, and produced photocurrent upon light irradiation. If the DNA film was exposed to a DNA-damaging chemical, photocurrent would drop due to the disruption of DNA double helical structure and consequently less binding of $\text{Ru}(\text{bpy})_2(\text{dppz})^{2+}$. Our results showed that the sensor was able to detect both oxidative DNA damage induced by $\text{Fe}^{2+}/\text{H}_2\text{O}_2$ and DNA adducts formed with styrene oxide.

In principle, this photoelectrochemistry DNA sensor is very sensitive, as the detected signal (electrical current) is separated from the signal generation source (light) [20,21]. To cite a few examples, Tokudome et al. constructed a DNA sensor by immobilizing a ss-DNA on TO_2 electrode and analyzing the complementary ss-DNA in solution. This sensor can quantitatively detect target DNA at lower than nanomolar concentrations [22]. Liu et al. employed the $\text{Ru}(\text{bpy})_2(\text{dppz})^{2+}$ metallointercalator and tin oxide nanoparticle electrode for the detection of ds-DNA in solution. The detection limit of calf thymus ds-DNA reached $1.8 \times 10^{-10} \text{ M}$ [23]. Gao and Tansil employed a threading DNA bis-intercalator as the photoelectrochemical reporter and achieved a dynamic range of 50 fM to 1.0 nM and a detection limit of 20 fM [24]. In addition, the instrumentation for electric detection should be simpler and of lower cost than optical methods. Thus, it is a promising method for the rapid and high-throughput assessment of DNA damage. However, due to the lack of appropriate platforms (sensor array and array detector), the potential of high throughput has not been demonstrated. It is the aim of the current work to develop microplate-based photoelectrochemical DNA sensors for the rapid and high throughput detection of DNA damage.

2. Material and methods

2.1. Materials

Fifteen percent tin (IV) oxide, as a colloidal dispersion of 15 nm particles, and hydrogen peroxide were both purchased from Alfa Aesar (Ward Hill, MA). Double-stranded calf thymus DNA (ds-DNA, approximately 13 K base pairs) was purchased from Merck (San Diego, CA). Poly-(diallyldimethyl ammonium chloride) (PDDA) and tetrafluoro-1,4-benzoquinone (TFBQ) were purchased from Sigma–Aldrich (St. Louis, MO). $\text{Ru}(\text{bpy})_2(\text{dppz})(\text{BF}_4)_2$ was synthesized according to the published procedure [25,26]. Indium tin oxide (ITO) conductive glass was supplied by Weiguang Corp (Shenzhen, Guangdong Province, China). All solutions were prepared in high purity water from a Millipore Milli-Q (Biocel) (Billerica, MA) water purification system.

2.2. Micro-plate fabrication and sensor assembly

Plastic substrates with an 8×12 electrode pair array were fabricated by a local manufacturer using the standard industrial procedures for the manufacturing of printed circuit boards. The electrode pair consists of a 2 mm diameter carbon disc electrode surrounded by an Ag/AgCl circular band (I.D. 4 mm, O.D. 7 mm) electrode. The footprint of the circular band matches that of a standard 96-well plate (Scheme 1). All the disc electrodes in the same line were electrically connected by a metal band on the backside of the substrate. So were the circular band electrodes in the same row.

To prepare a SnO_2 semiconductor electrode, typically the original colloidal dispersion of 15 nm SnO_2 particles was diluted to 3.75% with de-ionized water, and 0.1% Triton X-100 was added into the solution to facilitate the colloid spreading. A total of 0.25 μL diluted SnO_2 colloidal solution was then spread over the entire surface of each disc electrode. After the solution got dried naturally at room temperature, the film on the plastic substrate was sintered at 150°C in an oven for 2 h. Then, the substrate was attached to a bottomless 96-well plate (Greiner Bio-One GmbH, Germany) with a double-sided adhesive tape. The substrate and the plate were aligned carefully so that all the electrode pairs were exposed at the bottom of the 96 wells.

The layer-by-layer self-assembly method was employed to fabricate DNA sensor films on the SnO_2 electrode inside the

Scheme 1. Fabrication of a 96 electrode well plate. Conduction lines are fabricated on a plastic substrate by the standard printed circuit board manufacturing process. An array of 96 electrode pairs (carbon and Ag/AgCl) is processed by screen-printing. The board is then attached to a bottomless 96-well plate to finish the final assembly.

micro-well. For each well, 50 μL of 64 mg/mL PDDA (in 10 mM Tris buffer, pH 7.5) was added, and incubated for 16 h at room temperature. After rinse with water, 40 μL of 2 mg/mL DNA (10 mM Tris buffer, pH 7.5) was added into each well, and incubated for 2 h at room temperature. The plate was rinsed again with water.

2.3. DNA damage reaction and photocurrent measurement

TFBQ was dissolved in acetonitrile at a concentration of 150 mM and then diluted into 10 mM Tris-HCl (pH 7.5) for use. DNA damage reaction was performed by adding TFBQ alone or a mixture of TFBQ and H_2O_2 in Tris-HCl buffer (pH 7.5) into the micro-plate covered with DNA film, as described above. The reaction was allowed to proceed for 30 min at 37 $^\circ\text{C}$ before it was stopped and the plate was rinsed with water. The plate was then incubated with 25 μL of 50 μM $\text{Ru}(\text{bpy})_2(\text{dppz})^{2+}$ for 30 min at room temperature. Unbound metal complex was washed off with water, and 200 μL of 30 mM oxalate (pH 5.8) was added into each well for photoelectrochemical measurement. To measure the photocurrent of intact DNA sensor, the damage reaction was omitted, and $\text{Ru}(\text{bpy})_2(\text{dppz})^{2+}$ was reacted directly with the DNA film.

Photocurrent was measured on a home-made photoelectrochemical plate reader, which contains mainly a main control board, an X,Y-motion stage with a plate holder, a 470 nm photodiode, a current detection circuit board, and instrument software. There are

spring pins on the plate holder which make firm contact with the metal bands on the backside of the 96-well electrode plate and thus connect the electrodes to the instrument individually. During the measurement, each selected well of the plate was moved to a position under the photodiode, and a bias voltage of 0.1 mV was applied between the SnO_2 working and Ag/AgCl counter electrode. The photodiode was then turned on for 2 s, during which time photocurrent was recorded. Eight repeated wells were used for each set of experiments in the optimization of SnO_2 , DNA and $\text{Ru}(\text{bpy})_2(\text{dppz})^{2+}$ concentration, as well as in the investigation of DNA damage.

2.4. Film characterization

Scanning electron microscopy (SEM) images were taken on a JSM-6700F cold field emission SEM (JEOL Ltd., Tokyo, Japan). X-ray diffraction (XRD) spectra was recorded on an X'pert PRO XRD detector (PANalytical, Almelo, Netherlands) at a scan rate of 0.05°s^{-1} in the range from 20° to 80° . X-ray photoelectron spectroscopy (XPS) was carried out on a PHI Quantera SXM photoelectron spectrometer (ULVAC-PH Inc., Kanagawa, Japan) with a 0.5 eV binding energy resolution. A monochromatic Al K α X-ray radiation ($h\nu = 1486.7 \text{ eV}$) was used as excitation source under a pressure of $6.7 \times 10^{-8} \text{ Pa}$. The standard take-off angle used for analysis was 45° , producing a maximum analysis depth in the range of 3–5 nm.

Fig. 1. SEM images of screen-printed carbon electrode (A), SnO_2 nanoparticle film deposited on the carbon electrode and sintered at a temperature of 80 $^\circ\text{C}$ (B), 150 $^\circ\text{C}$ (C), and 200 $^\circ\text{C}$ (D). The white scale bar at the bottom of each image is 100 nm.

3. Results and discussion

Micro-plate based high throughput technologies have been developed and applied in the screening of chemicals as potential clinical drugs. These technologies typically employ absorbance, fluorescence and luminescence detection methods on 96-, 384-, or 1536-well plastic plates. The photoelectrochemical DNA sensor we previously developed offers a potentially high throughput technology for the screening of DNA-damaging chemicals. To realize its high throughput potential, micro-plates with built-in semiconductor electrodes are needed. For this purpose, a 96-well plate with a pair of electrodes built at the bottom of each well was designed, and manufactured by the established printed circuit board and screen printing processes due to their low cost and scale-up capability (Scheme 1). The electrode pair consists of a carbon working electrode and an Ag/AgCl reference/counter electrode. A tin oxide semiconductor electrode was then fabricated on top of the carbon electrode by forming a thick film of tin oxide nanoparticles from the corresponding SnO₂ colloid.

The semiconductor electrode is a vital part of the photoelectrochemical detection system. Typically, thick, porous semiconductor films are prepared by sintering nanoparticle colloids at relatively high temperatures (e.g., 450 °C). High temperature annealing is supposed to eliminate the organic reagents, binders and impurities remaining in the nanoparticle film. In addition, it also enhances the interconnection between particles and reduces the contact resistance [27]. However, the plastic substrate used for the 96-well plate is intolerant of such high temperatures. Therefore, SnO₂ films on the plastic substrate were prepared by annealing at temperatures below 250 °C, and their properties were investigated by a variety of techniques, as described below.

Fig. 1 is the SEM images of the carbon and SnO₂ film electrodes. The carbon film looks very rough and porous, with pore size in the range of a few hundred nanometers. Individual particles with a diameter of about 100 nm are clearly visible, and are mostly bound together to form larger-sized aggregates. In comparison, SnO₂ film covered surface appears much smoother and less porous. The porosity of the film depends on the annealing temperature, with the 200 °C annealed film the least porous. A closer examination of the SnO₂ film reveals that the particle size is in the range of 10–20 nm regardless of the annealing temperature.

The SnO₂ films were further characterized by XPS to investigate their chemical compositions. As shown in Fig. 2, when the annealing temperature was raised from 80 °C to 200 °C, the C_{1s} peak decreased and O_{1s} peak increased significantly. In the preparation of SnO₂ films, Triton X-100 (a surfactant) was added into the original SnO₂ colloidal solution to enhance nanoparticle dispersion. The XPS results suggest that, as the annealing temperature was raised, Triton X-100 and other organic components were removed gradually from the film, leading to a reduction in C_{1s} peak intensity. As the organic additives were removed from the surface, more SnO₂ particles were exposed, resulting in an increase in O_{1s} peak intensity.

It has been recognized that crystalline type of TiO₂ and other semiconductor nanoparticles greatly influences their photon to electron conversion efficiency [28]. Thus, XRD experiments were carried out to investigate the crystal structure of the SnO₂ films prepared at different temperatures. The films were prepared on ITO conductive glass substrates so that high temperature calcination could be performed. In Fig. 3, the ITO substrate showed two sharp and intense peaks at 30° and 35°, and two weak peaks at 51° and 60°. SnO₂ films deposited on ITO substrates produced three additional peaks at 26°, 34° and 52°. These three peaks were

Fig. 2. XPS C_{1s} and O_{1s} peaks of the SnO₂ nanoparticle films deposited on carbon electrode and sintered at a temperature of 80 °C, 150 °C and 200 °C.

Fig. 3. XRD spectra of bare ITO surface and SnO₂ nanoparticle films deposited on ITO and sintered at different temperatures.

in agreement with the standard diffraction spectra of rutile SnO₂ corresponding respectively to the (110), (101) and (211) plane [29,30]. They did not change much when the SnO₂ film was sintered in a temperature range from room temperature to 200 °C. However, when the temperature was raised to 450 °C, the intensity of the three SnO₂ peaks increased significantly. The XRD results indicate that the crystal structure of the SnO₂ nanoparticle film was similar at low calcination temperatures, but changed to more rutile structure at 450 °C.

After surface characterization, photoelectrochemical properties of the SnO₂ nanoparticle film electrode were investigated. On the film sintered at 80 °C, the photocurrent of dissolved Ru(bpy)₃²⁺ was very inconsistent. It was found that film adhesion to the substrate was very poor, and it peeled off easily when rinsed with water. Annealing at higher temperatures (150 °C and 200 °C) improved its adhesion and stabilized the photocurrent response. To minimize damage to the underlying plastic substrate, the annealing temperature was set to 150 °C. To optimize the film thickness, a range of SnO₂ colloid concentrations were tested. Fig. 4 shows the photocurrent signal of 5 μM Ru(bpy)₃²⁺ on the SnO₂ film electrode as a function of SnO₂ colloid concentration. The signal initially increased with SnO₂ concentration, then flattened out with 3.75% and 7.5% SnO₂, and finally dropped when the concentration reached 10%. Presumably, low concentration colloids did not produce a full film on the substrate, whereas the film deposited from 10% SnO₂ was too thick and not conductive enough. Although the signal average with 7.5% SnO₂ is a little higher than 3.75%, the difference is not significant by considering the variation of the signal. Therefore,

Fig. 4. Photocurrent of 5 μM Ru(bpy)₃²⁺ (in 30 mM oxalate, pH 5.8) measured on SnO₂ film electrodes in a 96-well plate with the film prepared from 1%, 1.9%, 3.75%, 7.5% and 10% SnO₂ colloids.

an optimum concentration of 3.75% was chosen in the following experiments.

The layer-by-layer self-assembly technique was employed to immobilize DNA on the SnO₂ film electrodes in 96-well plates, as reported before. The electrode surface was first covered with a layer of positively charged PDDA polymers. Then negatively charged DNA was assembled on the electrode by electrostatic interaction. Finally, Ru(bpy)₂(dppz)²⁺ was allowed to bind with the DNA film by intercalation and act as a photoelectrochemical signal reporter. It was found that PDDA not only presented a favorable surface for DNA immobilization, but also prevented Ru(bpy)₂(dppz)²⁺ from adsorbing directly on bare SnO₂ electrodes. As shown in Fig. 5A, a huge amount of photocurrent (1200 nA) was obtained on a bare SnO₂ electrode after it was in contact with 50 μM Ru(bpy)₂(dppz)²⁺ solution. However, once the electrode was covered with PDDA, the current diminished drastically. After incubating in 64 mg/mL PDDA solution, the current was reduced to less than 100 nA. On the electrode fully covered with PDDA, Ru(bpy)₂(dppz)²⁺ photocurrent demonstrated a strong dependence on DNA concentration, as shown in Fig. 5B. Comparing with the blank, the electrode assembled from a 2 mg/mL DNA solution produced six times more photocurrent. The photoelectrochemical DNA sensor thus assembled on the 96-well plate demonstrated good stability and reproducibility. The consistency of the microplate was evaluated by measuring the photocurrent of the DNA sensor assembled in each well of the whole microplate, using the optimized conditions (50 μL of 2 mg/mL PDDA, 40 μL of 2 mg/mL DNA, and 25 μL of 50 μM Ru(bpy)₂(dppz)²⁺). The test data showed that the relative standard deviation (RSD) of the photocurrent was less than 15% within each plate. And the RSD of the photocurrents from three plates was less than 10%. The microplate was then used to detect chemical-induced DNA damage in the following experiments.

In our previous work, photoelectrochemical DNA sensors fabricated on SnO₂-coated conductive glass were employed in the detection of DNA damages induced by halogenated quinones [17]. These compounds are the metabolites of halogenated phenols, an important class of environmental chemicals. They were found to covalently bind to DNA and form DNA adducts [4]. In addition, the halogenated quinones also generate hydroxyl radicals (HO•) in the presence of H₂O₂ and produce DNA oxidative damage [31,32]. Our sensor fabricated on the ITO glass was able to detect both types of DNA damages. To demonstrate the utility of the 96-well plate photoelectrochemical DNA sensor, tetrafluoro-1,4-benzoquinone

Fig. 5. A Photocurrent of non-specifically adsorbed Ru(bpy)₂(dppz)²⁺ on PDDA-covered SnO₂ film electrodes modified with different concentrations of PDPA solution. (B) Photocurrent of Ru(bpy)₂(dppz)²⁺ bound to DNA films on SnO₂ electrodes assembled from different concentrations of DNA solution. The photocurrent was measured in 30 mM oxalate, pH 5.8.

Fig. 6. Photocurrent of Ru(bpy)₂(dppz)²⁺ bound to DNA films on a 96-well plate after the DNA film was exposed to buffer, 300 μM TFBQ or 300 μM TFBQ/2 mM H₂O₂. The photocurrent was measured in 30 mM oxalate, pH 5.8.

(TFBQ) was tested as a model DNA-damaging agent. The results are shown in Fig. 6. After the DNA film was exposed to 300 μM TFBQ and then bound with Ru(bpy)₂(dppz)²⁺, average photocurrent was about 350 nA. This represents 38% reduction over the blank, and can be attributed to less amount of DNA-bound Ru(bpy)₂(dppz)²⁺ after TFBQ-DNA adduct formation. In the case of TFBQ/H₂O₂, the photocurrent decreased to 27% of the blank, mostly likely due to severe DNA damage by the highly reactive HO• radicals. In our previous work [33], the surface concentration of PDPA before and after it was exposed to the Fenton reagents was assessed by XPS, and no change was found. It suggests PDPA was not damaged by HO• radicals. The change of photocurrent measured on the 96-well plate after contact with TFBQ and TFBQ/H₂O₂ is very consistent with that observed on ITO glass. These results provide strong proof that the sensors fabricated on low-temperature sintered SnO₂ electrodes work equally well as those prepared on high-temperature annealed SnO₂ electrodes.

4. Conclusions

A 96-well microplate-based photoelectrochemical DNA sensor array was developed for the rapid and high throughput screening of DNA damaging chemicals. A low-temperature sintered SnO₂ nanoparticle film was prepared as the semiconductor electrode for the assembly of DNA sensor on the plastic plate. The SnO₂ film was characterized by SEM, XPS, XRD and photocurrent measurement. Although the films showed slightly different morphology, chemical composition, and crystal structure than the ones sintered at high temperatures, they exhibited acceptable photoelectrochemical properties. More importantly, the sensors assembled on the film detected successfully the DNA damage induced by a known chemical. Comparing with the single sensors fabricated on conductive glass, the plate-based sensor arrays can be used to assess a large number of chemicals in a rapid and high throughput fashion. On the home-built photoelectrochemical plate reader, detection of each sensor takes only 14 s. The plate and reader can also be used as a high throughput platform for the detection of a variety of biological binding assays such as DNA hybridization, antigen–antibody binding, and ligand–receptor interactions.

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References

- [1] P.D. Thacker, US companies get nervous about EU's REACH, *Environ. Sci. Technol.* 39 (2005) 171A–172A.
- [2] IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, International Agency for Research on Cancer, Lyon, France, 1980.
- [3] S. Kawanishi, Y. Hiraku, M. Murata, S. Oikawa, The role of metals in site-specific DNA damage with reference to carcinogenesis, *Free Radic. Biol. Med.* 32 (2002) 822–832.
- [4] W. Xue, D. Warshawsky, Metabolic activation of polycyclic and heterocyclic aromatic hydrocarbons and DNA damage: a review, *Toxicol. Appl. Pharmacol.* 206 (2005) 73–93.
- [5] G. Eisenbrand, B. Pool-Zobel, V. Baker, M. Balls, B.J. Blaauboer, A. Boobis, A. Carere, S. Kevekordes, J.C. Lhuguenot, R. Pieters, J. Kleiner, Methods of in vitro toxicology, *Food Chem. Toxicol.* 40 (2002) 193–236.
- [6] G.P. Pfeifer, Technologies for Detection of DNA Damage and Mutations, Plenum, New York, 1996.
- [7] J.-L. Ravanat, R.J. Turesky, E. Gremaud, L.J. Trudel, R.H. Stadler, Determination of 8-oxoguanine in DNA by gas chromatography-mass spectrometry and HPLC-electrochemical detection: overestimation of the background level of the oxidized base by the gas chromatography-mass spectrometry assay, *Chem. Res. Toxicol.* 8 (1995) 1039–1045.
- [8] P. Vodicka, K. Hemminki, ³²P-postlabeling of N-7, N² and O⁶ 2'-deoxyguanosine 3'-monophosphate adducts of styrene oxide, *Chem. Biol. Interact.* 77 (1991) 39–50.

- [9] R.C. Gupta, M.V. Reddy, K. Randerath, ^{32}P -postlabeling analysis of non-radioactive aromatic carcinogen–DNA adducts, *Carcinogenesis* 3 (1982) 1081–1092.
- [10] E. Palecek, M. Fojta, M. Tomschik, J. Wang, Electrochemical biosensors for DNA hybridization and DNA damage, *Biosens. Bioelectron.* 13 (1998) 621–628.
- [11] L. Zhou, J. Yang, C. Estavillo, J.D. Stuart, J.B. Schenkman, J.F. Rusling, Toxicity screening by electrochemical detection of DNA damage by metabolites generated in situ in ultrathin DNA-enzyme films, *J. Am. Chem. Soc.* 125 (2003) 1431–1436.
- [12] L. Dennany, R.J. Forster, J.F. Rusling, Simultaneous direct electrochemiluminescence and catalytic voltammetry detection of DNA in ultrathin films, *J. Am. Chem. Soc.* 125 (2003) 5213–5218.
- [13] K. Cahová-Kuchařiková, M. Fojta, T. Mozga, E. Paleček, Use of DNA repair enzymes in electrochemical detection of damage to DNA bases in vitro and in cells, *Anal. Chem.* 77 (2005) 2920–2927.
- [14] J. Liu, C. Roussel, G. Laguer, P. Tacchini, H.H. Girault, Antioxidant sensors based on DNA-modified electrodes, *Anal. Chem.* 77 (2005) 7687–7694.
- [15] Y. Zhang, N. Hu, Cyclic voltammetric detection of chemical DNA damage induced by styrene oxide in natural dsDNA layer-by-layer films using methylene blue as electroactive probe, *Electrochem. Commun.* 9 (2007) 35–41.
- [16] S. Jia, M. Liang, L.-H. Guo, Photoelectrochemical detection of oxidative DNA damage induced by Fenton reaction with low concentration and DNA-associated Fe^{2+} , *J. Phys. Chem. B* 112 (2008) 4461–4464.
- [17] S. Jia, B.-Z. Zhu, L.-H. Guo, Detection and mechanistic investigation of halogenated benzoquinone induced DNA damage by photoelectrochemical DNA sensor, *Anal. Bioanal. Chem.* 397 (2010) 2395–2400.
- [18] M.M. Liang, L.H. Guo, Photoelectrochemical DNA sensor for the rapid detection of DNA damage induced by styrene oxide and the Fenton reaction, *Environ. Sci. Technol.* 41 (2007) 658–664.
- [19] M.M. Liang, S.P. Jia, S.C. Zhu, L.H. Guo, Photoelectrochemical sensor for the rapid detection of in situ DNA damage induced by enzyme-catalyzed Fenton reaction, *Environ. Sci. Technol.* 42 (2008) 635–639.
- [20] R. Freeman, R. Gill, M. Beissenhirtz, I. Willner, Self-assembly of semiconductor quantum-dots on electrodes for photoelectrochemical biosensing, *Photochem. Photobiol. Sci.* 6 (2007) 416–422.
- [21] M. Li, R. Li, C.M. Li, N.Q. Wu, Electrochemical and optical biosensors based on nanomaterials and nanostructures: review, *Front. Biosci.* S3 (2011) 1308–1331.
- [22] H. Tokudome, S. Sonezaki, H. Ishikawa, M. Bekki, K. Kanehira, M. Miyauchi, Photoelectrochemical deoxyribonucleic acid sensing on a nanostructured TiO_2 electrode, *Appl. Phys. Lett.* 87 (2005) 213901.
- [23] S. Liu, C. Li, J. Cheng, Y. Zhou, Selective photoelectrochemical detection of DNA with high-affinity metallointercalator and tin oxide nanoparticle electrode, *Anal. Chem.* 78 (2006) 4722–4726.
- [24] Z. Gao, N.C. Tansil, An ultrasensitive photoelectrochemical nucleic acid biosensor, *Nucleic Acids Res.* 33 (2005) e123.
- [25] E. Amouyal, A. Homs, J.-C. Chambron, J.-P. Sauvage, Synthesis and study of a mixed-ligand ruthenium(II) complex in its ground and excited states: bis(2,2'-bipyridine)(dipyrido[3,2-a: 2',3'-c]phenazine- N^4N^5)ruthenium(II), *J. Chem. Soc. Dalton Trans.* 184 (1990) 1–1845.
- [26] S. Musumeci, E. Rizzarelli, I. Fraga, S. Sammartano, R.P. Bonomo, Low valence state of metal chelates. I. Complexes of iron(II) perchlorate with 1,10-phenanthroline, 4,7-dimethyl-1,10-phenanthroline and 4,7-diphenyl-1,10-phenanthroline, *Inorg. Chim. Acta* 7 (1973) 660–664.
- [27] A.M. Peiro, J. Peral, C. Domingo, X. Domenech, J.A. Ayllon, Low-temperature deposition of TiO_2 thin films with photocatalytic activity from colloidal anatase aqueous solutions, *Chem. Mater.* 13 (2001) 2567–2573.
- [28] N.G. Park, J. van de Lagemaat, A.J. Frank, Comparison of dye-sensitized rutile- and anatase-based TiO_2 solar cells, *J. Phys. Chem. B* 104 (2000) 8989–8994.
- [29] Z.R. Dai, J.L. Gole, J.D. Stout, Z.L. Wang, Tin oxide nanowires, nanoribbons, and nanotubes, *J. Phys. Chem. B* 106 (2002) 1274–1279.
- [30] J. Heo, A.S. Hock, R.G. Gordon, Low temperature atomic layer deposition of tin oxide, *Chem. Mater.* 22 (2010) 4964–4973.
- [31] B.Z. Zhu, N. Kitrossky, M. Chevin, Evidence for production of hydroxyl radicals by pentachlorophenol metabolites and hydrogen peroxide: a metal-independent organic Fenton reaction, *Biochem. Biophys. Res. Commun.* 270 (2000) 942–946.
- [32] B.Z. Zhu, B. Kalyanaraman, G.B. Jiang, Molecular mechanism for metal-independent production of hydroxyl radicals by hydrogen peroxide and halogenated quinones, *Proc. Natl. Acad. Sci. USA* 104 (2007) 17575–17578.
- [33] N. Qu, L.H. Guo, B.Z. Zhu, An electrochemical biosensor for the detection of tyrosine oxidation induced by Fenton reaction, *Biosens. Bioelectron.* 26 (2011) 2292–2296.

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