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Short communication

Rapid and sensitive electrochemical sensing of DNA damage induced by V_2O_5 nanobelts/HCl/H_2O_2 system in natural dsDNA layer-by-layer films

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ABSTRACT

The detection of DNA damage is one of the most important topics in the DNA research fields. In the present work, oppositely charged natural dsDNA and poly(diallyldimethyl ammonium chloride) (PDDA) were assembled into $(PDDA/dsDNA)_3$ layer-by-layer films on electrode surface, and $Ru(bpy)_3^{2+}$ and $Co(phen)_3^{3+}$ in solution were used as electroactive probes to detect oxidative damage of natural dsDNA in the films after incubation of the films in V_2O_5 nanobelts/HCl/H_2O_2 solution. The mechanism of DNA oxidative damage caused by the V_2O_5 nanobelts/HCl/H_2O_2 system was similar to that of Fenton-type reaction. The reaction of V_2O_5 nanobelts with HCl would produce V(IV), and the produced V(IV) would further react with H_2O_2, generating hydroxyl radicals ($OH^•$) as in the Fenton-type reaction, which could severely damage DNA in the films. The present work provided an in vitro model system to mimic the pathway of DNA damage in real bioprocess through a simple electrochemical approach combined with layer-by-layer assembly. This approach also showed promising applications in rapid and sensitive screening of new nanomaterials and chemicals in vitro for their potential genotoxicity.

1. Introduction

A growing concern in society regarding the possible adverse effects of manufactured nanoparticles has been raised in recent years. Due to the increased production and use of these particles in various products, consumer and occupational safety aspects must be considered as well as possible adverse effects on public health and ecosystems. Exposure to airborne particulate matter is known to induce different negative health outcomes, such as cardiovascular and pulmonary diseases and an increased risk of developing lung cancer. Examples of possible pathological pathways related to particle exposure, possibly leading to adverse health effects, are the generation of oxidative stress, inflammation, DNA damage, and cell death (Male et al., 2008; Tarantola et al., 2009; Jia et al., 2009).

In this context, nanotoxicology is an emerging discipline attempting to characterize and categorize the adverse effects caused by manufactured nanomaterials in order to determine structure/function relationships between nanoparticles and toxicity. These relationships will be used to formulate a set of design rules for the design of safe nanomaterials. The increased prevalence of these materials in both commercial goods and novel applications has brought nanotoxicology to the forefront and has called attention to the gap in toxicological information regarding these materials (Service, 2003; Lewinski et al., 2008; Miyawaki et al., 2008). Although there has recently been a tremendous amount of activities in this field, there are a large number of significant challenges that must be overcome to allow the safe incorporation of nanomaterials into commercial goods. From an analytical chemistry perspective, this emerging discipline poses many interesting challenges which will draw from both nanomaterial characterization and bioanalytical chemistry expertise within the field.

As one of the most functional biomolecules, DNA has received considerable attention among researchers, and the detection of DNA damage which is generally considered to be linked to mutagenesis, senescence, neurological and tumor formation has become one of the important scientific investigation fields (Yun et al., 2007; Rawle et al., 2008; Peng et al., 2008). Varieties of techniques have been proposed to investigate the DNA damage, such as the chromatography, electrophoretic separation, microbiological toxicity tests, as well as autoradiograms (Eisenbrand et al., 2002). However, all these techniques are time-consuming or laborious, or involve equipments with high cost. In recent years, electrochemical biosensors display obvious superiorities in detecting DNA damage with their simplicity, relatively cheap cost, fast response and low power requirement, and therefore have aroused great interests among researchers (Zhang et al., 2009; Havran et al., 2008; So et al., 2008;

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Zu and Hu, 2009; Wang et al., 2009). Oxidants, particularly reactive oxygen species (ROS), are regarded as main DNA oxidative damage reagents. Among ROS, hydroxyl radicals ($OH^{\bullet}$) can attack DNA molecule and play a major role in the formation of DNA oxidative damage. Guanine is the most easily oxidized DNA base, mainly producing 8-hydroxydeoxyguanosine (8-OHdG), and damage to DNA also has the effect of unwinding the double helix (Huang et al., 2008).

In this work, layer-by-layer films of negatively charged natural dsDNA and polycationic poly(diallyldimethyl ammonium chloride) (PDDA) were assembled on the glassy carbon electrode (GCE) surface, forming $(PDDA/dsDNA)_3$ films. After the films were incubated in the V_2O_5 nanobelts/HCl/H_2O_2 system, $OH^{\bullet}$ was produced through a series of reactions and would induce the oxidative damage of DNA in the films. The DNA damage could then be detected by cyclic voltammetry (CV) with $Ru(bpy)_3^{2+}$ and $Co(phen)_3^{3+}$ as the electroactive catalysts in solution. To the best of our knowledge, the electrochemical sensing of DNA oxidative damage induced by nano-V_2O_5 has not been reported up to now. This work provides a novel in vitro working model system that can be used to imitate the DNA damage process in vivo.

2. Experimental

2.1. Apparatus

All the electrochemical measurements were performed on a CHI 660C electrochemical workstation (Shanghai CH Instrument Company, China). A conventional three-electrode system was used with a modified GCE as working electrode, a saturated calomel electrode (SCE) as reference electrode and a platinum wire as auxiliary electrode. Scanning electron microscopy (SEM) was carried out using a JSM-6700F machine (JEOL, Tokyo, Japan).

2.2. Chemicals

V_2O_5 nanobelts were synthesized as reported previously (Li et al., 2006) and dispersed in ethanol to 2.0 mg/mL. In brief, 0.36 g of commercial V_2O_5 powders was added to 60 mL of ultrapure water with shaking to form a light yellow slurry solution. Then, 5 mL of 30% H_2O_2 was added dropwise to the slurry solution and stirred for 5 min to form an orange solution. The orange solution was placed in a 100 mL autoclave with a Teflon liner. The autoclave was maintained at 180 °C for 48 h and then was air cooled to room temperature. It was found that flocculent precipitates existed in the solution. The resulting precipitates were collected and washed with ultrapure water several times and then dried in a vacuum at 60 °C for 10 h.

Herring sperm dsDNA, PDDA and $Ru(bpy)_3Cl_2{\bullet}6H_2O$ were purchased from Sigma. $[Co(phen)_3](ClO_4)_3$ was prepared in our lab and dissolved in 10 mM phosphate buffer (pH 7.0) to 100 μM. All other chemicals were of analytical grade and ultrapure water was used in all the experiments.

2.3. Procedures

For electrochemical studies, the layer-by-layer films were assembled on the GCE surface. In brief, the clean GCE was alternately immersed in the positively charged PDDA solution (1.0 mg/mL, containing 0.1 M NaCl) and the negatively charged dsDNA solution (1.0 mg/mL, in 5.0 mM Tris–HCl buffer at pH 7.0, containing 0.1 M NaCl) for 20 min, forming $(PDDA/dsDNA)_3$ multilayer films.

For DNA damage by V_2O_5 nanobelts/HCl/H_2O_2 system, 25 μL of 2.0 mg/mL V_2O_5 nanobelts and 2 mL of 5.0 mM HCl were first mixed for 5 min, and 2 mL of 5.0 mM H_2O_2 was then added right before the incubation. The $(PDDA/dsDNA)_3$ films were incubated in the V_2O_5 nanobelts/HCl/H_2O_2 solution at 37 °C with stirring for a certain time. The damaged films were then washed with ultrapure water and transferred into pH 7.0 phosphate buffer containing 50 μM $Ru(bpy)_3^{2+}$ or 100 μM $Co(phen)_3^{3+}$ for cyclic voltammetric (CV) scans.

3. Results and discussion

3.1. Morphological characterization of the synthesized V_2O_5

Fig. 1A and B shows typical SEM images of the synthesized V_2O_5. As shown in Fig. 1A, the products are composed of a large quantity of one-dimensional nanobelts with lengths up to several micrometers. In a high-magnification SEM image (Fig. 1B), it is clear that a few V_2O_5 nanobelts can assemble into bundle-like nanostructures. The widths and thickness of the nanobelts are in the range of 100–300 nm and 20–30 nm, respectively.

3.2. Electrochemical detection of DNA damage induced by V_2O_5 nanobelts/HCl/H_2O_2 system

3.2.1. $Ru(bpy)_3^{2+}$ as the probe

For PDDA film electrode, the CV of $Ru(bpy)_3^{2+}$ in pH 7.0 phosphate buffer showed an obvious oxidation peak at about 1.00 V, characteristic of the electrochemical oxidation process of

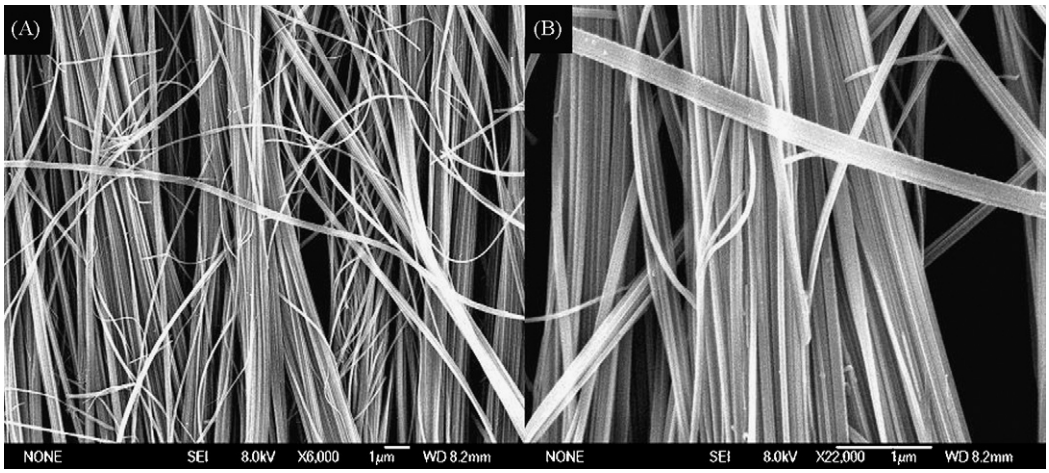

Fig. 1. (A) Low-magnification and (B) high-magnification SEM images of the synthesized V_2O_5.