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Short communication

Quantum dot-based electrochemical DNA biosensor using a screen-printed graphite surface with embedded bismuth precursor

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ABSTRACT

This work reports the development of screen-printed quantum dots (QDs)-based DNA biosensors utilizing graphite electrodes with embedded bismuth citrate as a bismuth precursor. The sensor surface serves both as a support for the immobilization of the oligonucleotide and as an ultrasensitive voltammetric QDs transducer relying on bismuth nanoparticles. The utility of this biosensor is demonstrated for the detection of the C634R mutation through hybridization of the biotin-tagged target oligonucleotide with a surface-confined capture complementary probe and subsequent reaction with streptavidin-conjugated PbS QDs. The electrochemical transduction step involved anodic stripping voltammetric determination of the Pb(II) released after acidic dissolution of the QDs. Simultaneously with the electrolytic accumulation of Pb on the sensor surface, the embedded bismuth citrate was converted in situ to bismuth nanoparticles enabling ultra-trace Pb determination. The biosensor showed a linear relationship of the Pb(II) peak current with respect to the logarithm of the target DNA concentrations from 0.1 pmol L^{-1} to 10 nmol L^{-1}, and the limit of detection was 0.03 pmol L^{-1}. The biosensor exhibited effective discrimination between a single-base mismatched sequence and the fully complementary target DNA. These "green" biosensors are inexpensive, lend themselves to easy mass production, and hold promise for ultrasensitive bioassay formats.

1. Introduction

Among the various DNA biosensors (i.e., devices that embody both the biorecognition and the transducer components into a single package), electrochemical biosensors offer unique advantages in terms of sensitivity, selectivity, working simplicity and, thus, are considered a viable alternative to expensive and labor-intensive commercially available methods [1,2]. In particular, exploiting quantum dots (QDs) labels in combination with anodic stripping voltammetry (ASV) for their detection allows the design of ultrasensitive DNA assays via selection of a proper combination of QDs core type and electrode material [1,2]. Nevertheless, in the existing ASV-QD DNA assays, the surfaces on which the DNA assay is performed cannot be used as sensitive transducers for the ASV determination of the metallic ions released from QD labels; therefore, the hybridization/labeling step and the detection step take place at spatially separated surfaces. More specifically, in these applications the biorecognition events are conducted in microwells [3,4], on glass substrates [5], on magnetic beads [6–9], on carbon nanotubes [10], or on gold surfaces [11–13], while the ASV detection step of QDs is carried out at mercury- or bismuth- film sensors prepared via electroplating procedures on rigid glassy carbon electrodes. Besides the existing methodologies involve an additional step for generating the voltammetric detection layer while the electroplated metal-film electrodes present several drawbacks associated with the creation and deposition reproducibility of the Hg or Bi film. In order to address these limitations of electroplated transducers, sputtered Sn and Bi sensors have been applied to the ASV-QD DNA analysis after performing the DNA assays in microwells [14,15]. Still, the aforementioned ASV-QD DNA sensors fail to offer the simplicity and degree of integration expected from electrochemical biosensors.

In this work, we report the fabrication of disposable, quantum dot-based DNA biosensors developed on a screen-printed graphite surface with embedded bismuth citrate as a bismuth precursor. Proof-of principle applicability is demonstrated for the ASV-QD assay of the C634R mutation of the RET gene (related to Multiple Endocrine Neoplasia Type 2 (MEN2)). The operation of these electrochemical biosensors is based on (i) the DNA assay onto their surface, involving hybridization of the biotinylated target oligonucleotide with the surface-immobilized complementary probe and its labeling with streptavidin-conjugated PbS QDs and (ii) the ASV detection of acidically released Pb(II) from QDs at in situ formed Bi nanoparticles on the same surface (as embedded bismuth citrate is reduced to Bi simultaneously with the electrolytic accumulation of Pb). The in situ generation of the Bi nanoparticles renders the biorecognition graphite surface an ultrasensitive voltammetric QDs

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transducer. Consequently, at the proposed QD-based biosensors, both the hybridization/labeling step and the voltammetric detection step are combined at the same surface, in contrast to the existing ASV-QD DNA detection methodologies. Moreover, since the generation of the bismuth nanostructured film occurs in situ by the reduction of the bismuth precursor embedded in the electrode, addition of Bi(III) or Hg(II) in the sample solution is not required, thus simplifying the assay workflow.

2. Experimental

2.1. Reagents and apparatus

Bovine serum albumin (BSA) was purchased from Pierce, while the oligonucleotides were from VCB Biote. The 5′-end amine-tagged capture complementary oligonucleotide was 5′-CGACGAGCTGTGCCGC ACGGT-3′, and its conjugation with BSA was carried out according to the literature [15]. The 5′-end biotin-tagged target DNA sequence was 5′-ACCGTGCGGCACAGCTCGTCG-3′, and the 5′-end biotinylated non-complementary and the 5′-end biotinylated single-base mismatch DNA sequences were 5′-TGGCGTACTCCACGATGAGGA-3′ and 5′-ACCG TGCGGCGCAGCTCGTCG-3′, respectively. The PbS QDs were synthesized and conjugated with streptavidin (STV) according to our previous work [15]. A field emission scanning electron microscopy (FESEM) (JEOL JSM-7401f) image of the STV-PbS QDs is shown in Fig. 1A.

The electrochemical experiments were carried out in a 1 mL electrochemical cell using a PGSTAT101 (Metrohm Autolab) potensiostat. The NOVA 1.8 software (Metrohm Autolab) was used for the baseline correction of the voltammograms. The surface structure of the biosensors was investigated with a JSM-6510LV scanning electron microscope equipped with an INCA Penta FET˝x3 energy dispersive X-ray (EDX) spectroscopy detector (Oxford Instruments).

2.2. Fabrication of devices

Screen-printed three-electrode cells (SPCs) were fabricated onto a flexible polyester sheet (0.175 mm thick, CUS7, Mac Dermid) using a semi-automatic screen printer (DEK 247), stainless steel screens (230 mesh, emulsion thickness 13 μm), and a 75 durometer polyurethane squeegee. SPCs were consisted of four layers printed in the following order: (i) a layer made of silver (PF-410, Acheson) that acts as the conductive track for the working and the auxiliary electrodes, and as the reference electrode; (ii) a graphite layer (PF-407A, Acheson) that serves as auxiliary electrode; (iii) the working layer, which produced by mixing 9.4 g of the graphite ink and 0.6 g bismuth citrate; and(iv) a dielectric layer (D2000222D2, Gwent). Each layer was

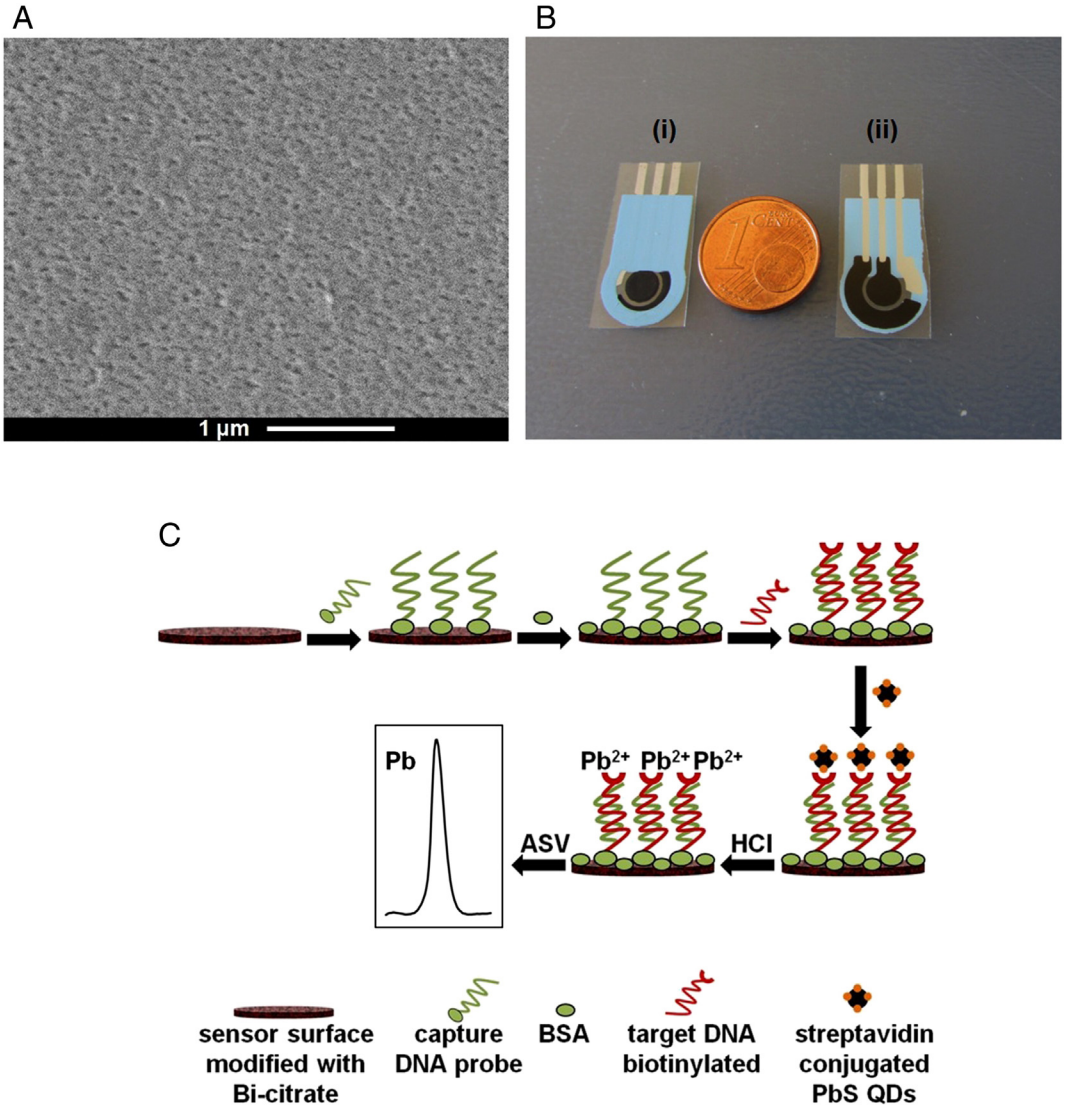

Fig. 1. (A) FESEM image of STV-PbS QDs. (B) Photograph of the devices: (i) front and (ii) back face. (C) Illustration of the DNA assay developed on the biosensor surface.