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Short communication

Streptavidin functionalized nickel nanowires: A new ferromagnetic platform for biotinylated-based assays

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ABSTRACT

Herein we present highly stable ferromagnetic nickel nanowires modified with streptavidin (NiNW-STR). This versatile functionalized nanomaterial works as an excellent biosensing platform for the immobilization of a wide range of biotinylated molecules. Moreover, these NWs can be employed in magnetic-based assays. Different proofs-of-concept such as streptavidin–biotin assays and capture of single and double stranded DNA were successfully carried out, corroborating NiNW-STR usefulness. Moreover, repeatability and stability studies were also effectively performed.

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1. Introduction

Nowadays, the advances in nanotechnology opened new opportunities to explore modern analytical applications. Among them, metal-based nanowires (NWs) are attracting great attention as building blocks for sensing devices in chemistry and biochemistry [1]. NWs, with their one-dimensional (1D) morphology and extraordinary physical and chemical properties capture the attention of the scientific community due to their improved functionalities compared to the bulk material [1,2]. As electrochemical techniques are surface dependent, the introduction of NWs contributes to the improvement of the electroanalytical performance [3]. Advantages such as lower detection potentials, higher stability, resistance to electrode passivation, higher analytical sensitivity, and high compatibility and functionality with biomolecules are attributed to the use of NWs in the electrochemical sensing and biosensing field [3]. NWs made from different materials (metal, metal oxides or polymers) have been widely described [2–4]. Particularly, nickel nanowires (NiNWs), due to their intrinsic magnetization that allows magnetic manipulations through the application of an external magnetic field, present great potential for electrochemical biosensing [5–9]. Hence, when the potential features of the NWs are combined with the selectivity and the sensitivity of biomolecules, the possibilities of these bio-hybrid structures are enormous. Streptavidin coated-surfaces have been extensively applied to solid-phase assays, due to the extraordinary properties of streptavidin–biotin interaction [10]. For this reason, and to the fact that streptavidin–biotin linkage is extremely tight, specific and stable [11], NiNWs functionalised with streptavidin (NiNW-STR) can work as an universal platform for magnetic-based capture of biotin-labeled molecules (*e.g.*, cells, proteins, and nucleic acids) in a variety of assays, allowing an oriented immobilization on the surface.

Therefore, combining the advantages of streptavidin modified platforms with the ferromagnetic properties of NiNWs, nickel nanowires functionalized with streptavidin (NiNW-STR) were developed. This innovative and highly-stable bio-functionalized nanomaterial is an excellent option for the immobilization or capture of a large amount of biotinylated molecules. Moreover, due to nickel ferromagnetic behavior, these NWs are a perfect candidate to be use in magnet-based (bio)assays.

2. Experimental

2.1. Instrumentation

Voltammetric measurements were performed with a portable bipotentiostat/galvanostat μSTAT 400 (DropSens; Spain) potentiostat interfaced to a Intel(R) Core(TM)2 Duo CPU computer

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system and controlled by DropView 8400 2.1 software. All measurements were carried out at room temperature (RT). Disposable screen-printed carbon electrodes (DRP-110; DropSens) were employed as the electrode surface. The DropSens' electrodes incorporate a conventional three-electrode cell configuration, printed on ceramic substrates (dimensions: $3.4 \times 1.0 \times 0.05$ cm (length × width × height). Both working (disk-shaped 4 mm diameter) and counter-electrodes are made of carbon inks, whereas pseudoreference electrode and electric contacts are made of silver. An insulating layer was printed over the electrode system, leaving uncovered the electric contacts and a working area which constitutes the reservoir of the electrochemical cell, with an actual volume of 50 μL. The SPEs were easily connected to the potentiostat through a specific DropSens connector (DRP-DSC). A microcentrifuge tubes support (DRP-MAGNET16TUBE15) for the magnetic isolation/separation of nickel nanowires (NiNWs) and magnetic support for SPCEs (DRP-MAGNET) were provided by DropSens. A vortex mixer (Vortex Scientifica; Spain) was also used. A Leica DMIRE2 inverted microscope was employed to characterize NiNWs modified with streptavidin.

2.2. Reagents and solutions

Tris(hydroxymethyl)aminomethane (Tris), sodium phosphate dibasic, potassium phosphate dibasic, magnesium nitrate, bovine serum albumin fraction V (BSA), N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC), N-Hydroxysulfosuccinimide sodium salt (sulfo-NHS), streptavidin (molecular weight, 60 kDa), and anti-fluorescein isothiocyanate (FITC) antibody labeled with AP (anti-FITC-AP) were purchased from Sigma-Aldrich (Spain). Biosynth (Switzerland) supplied 3-indoxyl phosphate disodium salt (3-IP). Silver nitrate, nitric acid, sodium hydroxide, hydrochloric acid, sodium chloride, and potassium chloride were obtained from Merck (Spain). Biotin conjugated to alkaline phosphatase (B-AP; dimer, four units of B per molecule of AP, molecular weight, 140 kDa) was acquired to Thermo Scientific (Spain). All oligonucleotides used were purchased to Invitrogen (Spain). The buffer solutions more employed through this work were: 0.1 M Tris–HNO_3 pH 7.2 (buffer 1); 0.1 M Tris–HNO_3 pH 7.2 containing 2 mM $Mg(NO_3)_2$ (buffer 2); 0.1 M Tris–HNO_3 pH 9.8 containing 20 mM $Mg(NO_3)_2$ (buffer 3); phosphate saline buffer (PBS) with 0.5% BSA and PBS with 0.1% BSA. Working solutions of streptavidin and oligonucleotides were made with buffer 1. Working solutions of B-AP and anti-FITC-AP were prepared in buffer 2. Solutions of 1.0 mM 3-IP and 0.4 mM silver nitrate were prepared daily in buffer 3 and stored in opaque tubes at 4 °C. Ultrapure water obtained with a Millipore Direct-QTM purification system from Millipore Ibérica S.A. was used throughout this work. All chemicals employed were of analytical reagent grade.

2.3. Fabrication of NiNWs

Ni nanowires ($\sim 9 \times 10^9$ NiNWs; 7 μm long) were synthesized by potenciostatic electrodeposition method using a porous anodic alumina oxide membrane (AAO), with cylindrical nanopores of 200 nm in diameter, as template for nanowire growth. AAO was sputtered with gold in its branched side to act as the working electrode during the electrodeposition (in connection to an aluminum foil contact). Ag/AgCl and Pt wires were used as reference and counter electrodes, respectively. Nickel was deposited into the pores by electroplating from an Watts-type bath with 300 g/L $NiSO_4 \cdot 6H_2O$, 45 g/L $NiCl_2 \cdot 6H_2O$ and 45 g/L H_3BO_3 at 35 °C, pH 4–4.5, and applying a deposition potential in the range of −1.2 V *versus* the reference electrode. Afterwards, in order to release the NiNWs from the template, a chemical etching procedure was carried out. Firstly, the gold layer was partially removed by wet chemical etching in KI 0.6 M and I_2 0.1 M aqueous solution and, afterwards, the alumina membrane, was dissolved by immersion in a mixture of H_3PO_4 (6 wt%) and CrO_3 (1.8 wt%) at 45 °C for 48 h. Once in suspension, the NWs were first filtered, and subsequently, transferred to a microcentrifuge tube where were dispersed in ethanol.

2.4. Covalent functionalization of NiNWs with streptavidin

NiNWs ($\sim 1 \times 10^9$ NiNWs) surface was modified with streptavidin through a covalent linkage *via* carbodiimide chemistry, forming a highly stable amide bond between carboxylic acid coated NiNWs and amino groups of streptavidin. Carboxylic groups were introduced in the surface of NiNWs by performing a previous treatment with a dycarboxilic acid for 24 h at room temperature (RT). For the carboxyl-to-amine crosslinking, EDC 2 mM and sulfo-NHS 5 mM were employed, in the presence of streptavidin 4 μM and the reaction carried out for 5 h, at RT, with gentile stirring of the NiNWs and reagents. After that, NiNW-STR were washed twice with 0.5% BSA in PBS and, finally, resuspended in 0.1% BSA in PBS and stored at 4 °C. The final NiNW-STR concentration was ~9.795 mg/mL. A neodymium magnet was used in all the washing and separation steps.

2.5. Streptavidin–biotin affinity assay

The streptavidin-modified NiNWs (40 μL of NiNWs-STR; $\sim 8 \times 10^7$ NiNWs) were employed in the study of streptavidin–biotin affinity interactions. NiNW-STR were incubated with different concentrations of B-AP for an hour reaction at RT with gentle and continuous agitation (500 rpm). After three washing steps (buffer 3), 35 μl of the final reaction product were transferred to a carbon screen-printed electrode surface (DRP-110) covering the three-electrode cell. The pre-concentration of the NWs onto the electrode surface was carried out by using a neodymium magnet, placed under the working electrode (DRP-MAGNET). After 1 min, 15 μL of AP enzymatic substrate (1 mM 3-IP/0.4 mM Ag^+) was added. AP hydrolyzes 3-IP resulting in an indoxyl intermediate that will reduce the silver ions presents in solution resulting in metallic silver (Ag°) and indigo blue. Thus, the silver enzymatically deposited on the electrode surface can be detected through the anodic peak of the silver when an anodic stripping scan is carried out [13]. Therefore, after 2 min of enzymatic reaction a linear sweep voltammogram (LSV) was recorded from −0.02 V to +0.4 V, at scan rate of 50 mV s^{-1}, and the redissolution peak of the silver deposited on the electrode surface was recorded. The described procedure was also applied in stability and repeatability studies. In these cases, a concentration of 1000 pM was the B-AP concentration assayed.

2.6. Capture of a biotinylated ssDNA with NiNW-STR

2.6.1. Oligonucleotide sequence

5′-CAAACACCATTGTCACACTCCA-3′, labeled with FITC at 5′ and biotin at 3′.

40 μL of NiNW-STR ($\sim 8 \times 10^7$) were incubated with 100 μL of FITC-5′-Oligo-3′-Biotin (1 μM) for 30 min at RT with gentle and continuous agitation (500 rpm). After a washing step (buffer 1), NiNW-STR conjugated to Biotin-3′-Oligo-5′-FITC were resuspended in 50 μL of anti-FITC-AP (1:12,500). The reaction was left to proceed for another 30 min at RT with continuous agitation (500 rpm). After a second washing step (Buffer 3) the final reaction product was resuspended in 110 μL of Buffer 3 and 35 μL of the final product was transferred to the SPCE surface (with a neodymium magnet (DRP-MAGNET) under the working electrode). Following to 1 min of pre-concentration, 15 μL of a mixture of 1.0 mM

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