Short communication

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

M.M.P.S. Neves*, M.B. González-García, D. Hernández-Santos, P. Fanjul-Bolado

DropSens, S.L., Edificio CEEI, Parque Tecnológico de Asturias, 33428 Llanera, Asturias, Spain

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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 bipotentiotstat/galvanostat µSTAT 400 (DropSens; Spain) potentiostat interfaced to a Intel(R) Core(TM)2 Duo CPU computer.
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 × 1.0 × 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 electronic contacts are made of silver. An insulating layer was printed over the electrode surface, 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 potential stat 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, 140 kDa) was acquired to Thermo Scientific (Spain). Biosynth (Switzerland) supplied 3-indoxyl phosphate (3-IP), Ni nanowires (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 I2 0.1 M aqueous solution and, afterwards, the alumina membrane, was dissolved by immersion in a mixture of H3PO4 (6 wt%) and CrO3 (18 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 (~1 × 10⁹ NiNWs) surface was modified with streptavidin through a covalent linkage via carbodiimide chemistry, forming a highly stable amidic 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 gentle 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; ~8 × 10⁷ 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

Ni nanowires (~9 × 10⁹ 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 NiSO₄ 6H₂O, 45 g/L NiCl₂ 6H₂O and 45 g/L H₃BO₃ 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

2.6.1. Oligonucleotide sequence

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5'-\text{CAACAGCTGGTACACTCCA-3}, \text{ labeled with FITC at } 5' \text{ and biotin at } 3'.
\]

40 μL of NiNW-STR (~8 × 10⁹) 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 washing with 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
3-IP/0.4 mM Ag⁺ were added and used as the AP substrate. After 5 min of enzymatic reaction a LSV was recorded, as described in Section 2.5.

2.7. Capture of a dsDNA with NiNW-STR

2.7.1. Probe oligonucleotide sequence

5′-CAACACCATGTGACCTCCA-3′ (labeled with biotin at 5′)

2.7.2. Target oligonucleotide sequence

5′-TGGAGTGTGACAATGGTGTTTG-3′ (labeled with FITC at 5′)

40 μL of NiNW-STR (∼ 8 × 10⁴) previously modified with biotin-labeled probe were incubated with FITC-labeled target (50 μL; 1 μM) for 30 min at RT with continuous agitation (500 rpm). After another washing step (buffer 3) the final reaction product was resuspended in 110 μL of buffer 3. 35 μL of the final product was transferred to the SPCE surface (with a neodymium magnet (DRP-MAGNET) under the working electrode). After 1 min of NWs pre-concentration, 15 μL of a mixture of 1.0 mM 3-IP/0.4 mM Ag⁺ were added and used as the AP substrate. After 10 min of enzymatic reaction a LSV was recorded, as described in Section 2.5.

3. Results and discussion

Ni nanowires (7 μm long) were synthesized by electrodeposition using a porous alumina template. The preparation of highly ordered nanostructures with specific dimensions and properties was controlled through the charge recorded during the nanowire growth [12]. Afterwards, streptavidin was covalently coupled to the oxidized NiNW surface, via carbodiimide chemistry, employing EDC (carbodiimide) plus sulfo-NHS crosslinking reaction. The functionalization procedure did not affect the ferromagnetic properties of the NiNWs. Different proofs-of-concept were carried out in order to determine the versatility and potential of NiNW-STR.

3.1. Streptavidin–biotin affinity assay

The streptavidin-modified NiNWs were employed in the study of streptavidin–biotin affinity interactions. The obtained voltammograms are shown in Fig. 1. All the voltammograms corresponded to the analytical signal achieved with NiNW-STR to different concentrations of B-AP, except for the black dashed line voltammogram which was obtained with non-modified NiNWs in the presence of 1000 pM of B-AP. Linear range from 0 to 100 pM, with a LOD (as the minimum B-AP concentration detected) of 10 pM, for a 2 min enzymatic reaction, and without any blocking step in the bioassay, indicates the high sensitivity and selectivity of this biotin-capture nanosurface. Besides, the obtained data indicates that NiNW-STR can be of use for fast response type assays.

3.2. Stability and inter- and intra-repeatability studies

Employing the same procedure (Section 2.5), the stability and functionality of streptavidin functionalized NiNWs was assayed for a 6 months period. For that purpose, NiNW-STR were prepared and stored at 4 °C. The assay was carried out past 7, 15, 30, 60, 90 and 180 days from the NiNWs-STR production, and using three replicates for each measurement. The results (data not shown), with an average relative standard deviation (RSD) around 5%, did

![Fig. 1. Linear sweep voltammograms corresponding to the response of the NiNW-STR to growing concentrations of Biotin-AP. Experimental conditions: 3-IP 1 mM; Ag⁺ 0.4 mM. Voltammetric scans from -0.02 V to +0.4 V at a scan rate of 50 mV s⁻¹. Inset: Linear relationship between anodic peak current intensity and the Biotin-AP concentration (average data ± standard deviation are indicated (n=3)).](image-url)
Fig. 2. Confocal microscopy images of NiNW-STR modified with an oligonucleotide labeled with FITC at 5’-end and biotin at 3’-end without (a) and with (b) the presence of an external magnetic field. Comparison of confocal (c1 and d1) and transmission electron microscopy (c2 and d2) images of NiNW-STR (c) and NiNWs (d), both previously incubated with the referred oligonucleotide.
not indicate any appreciable change among the analytical signal. Moreover, inter- and intra-repeatability in the production of three different NiNW-STR batches were also verified. Using the procedure indicated above, RSD (%) values less than 2% were obtained using three replicates for measurement of each batch assayed, indicating that these functionalized NiNWs are produced under a highly reproducible and controlled process. Therefore, NiNW-STR are a stable high affinity nanosurface for the immobilization of a large amount of biotinylated molecules, providing a versatile platform for the development of several (bio)sensors.

3.3. Microscopic characterization of NiNW-STR

After confirm the affinity interaction between NiNW-STR and biotinylated molecules, these NWs were incubated with a 22-mer 3′-end biotinylated ssDNA also labeled with fluorescein at 5′-end (FITC-5′-Oligo-3′-Biotin). After 30 min of incubation, the product of the reaction was observed with confocal and transmission electron microscopy in order to assess the efficiency of protein-binding (Fig. 2). STR functionalized NiNWs exhibited the characteristic green fluorescence of FITC (Fig. 2a–c1). Moreover, the obtained images (b) reveal that, in general, when a magnetic field is applied, the NiNW-STR orient themselves perpendicular to the magnetic field axis, according to previous reports [14,15]. Furthermore, the comparison of confocal and transmission images indicates that streptavidin-modified NWs were fully covered with the FITC-label, which indicates that the affinity reaction was successfully achieved (Fig. 2c1 and c2). On the other hand, non-modified NiNWs (without streptavidin), and also incubated with FITC-5′-Oligo-3′-Biotin, do not exhibit any fluorescence (Fig. 2d1 and d2).

3.4. NiNW-STR genosensing assays

This microscope results were also supported by the electrochemical data obtained (Fig. 3a). For the electroanalytical assay, an anti-FITC antibody labeled with AP was employed as detection element and incubated for 30 min with FITC-5′-Oligo-3′-Biotin linked to NiNW-STR. Afterwards, the NiNW-STR were also applied as solid surface platforms for the immobilization of a dsDNA. For that purpose, the NWs previously modified with the 3′-end biotinylated oligonucleotide were used as capture probe for a complementary target sequence which was 5′-end FITC tagged (Fig. 3b). Electroanalytical responses of the described proofs-of-concept are shown in Fig. 3. The results confirm that NiNW-STR are a highly specific platform, with no significant background or either non-specific bindings (NSB), even without employing any blocking agent in the bioassay and in the presence of considerable size proteins, such as antibodies and enzymes. This fact can be attributed to the NiNW-STR specific properties. In one hand, due to the surface be fully coated with STR and, as a consequence, effectively blocked, minimizing possible NSB and, in the other hand, to the magnetic properties, which contribute to an effective wash of the NWs surface, before the immobilization of the product of reaction in the electrode, allowing the electrode to work only as transducer surface and, consequently, avoiding electrode surface fouling.

4. Conclusions

In summary, we present a novel bioconjugated nanosurface. Ferromagnetic nickel nanowires prepared on AAO template were functionalized with streptavidin. The effective immobilization of biotinylated molecules was successfully demonstrated and this new NiNW-STR were effectively applied in the detection of a 22-mer oligonucleotide. This functionalized nanomaterial was also characterized by confocal and transmission electron microscopy. The efficient coating of nickel nanowires with streptavidin as well as the excellent control of NiNW-STR by applying a magnetic external field was verified. NiNW-STR appears as an exciting nanomaterial that can be applied within a wide range of areas, such as chemistry and electrochemistry, biochemistry, immunology, cell and molecular biology. NiNW-STR can be used to isolate proteins, cells, DNA as well as be used in immunoassays. Moreover, due to their magnetically controlled driven these NWs can result useful as “nanoengines”.

Fig. 3. (a) Immobilization of an oligonucleotide labeled with FITC at 5′-end and biotin at 3′-end on the NiNW-STR (A). Evaluation of the non-specific adsorptions (B–D); current measured in the absence of a magnetic field under the WE surface (B), with non-modified NiNWs (without STR) (C) and with a non-labeled oligonucleotide (D). (b) Capture of a dsDNA with NiNW-STR (capture probe: Biotin-5′-Oligo-3′); Complete assay (A); i_p = +0.18 V. Evaluation of the non-specific adsorptions (B–D); current measured with non-modified NiNWs (without STR) (B), in the absence of the capture probe (C) and in the absence of the labeled target (D); average data ± standard deviation are indicated (n = 3). Experimental conditions (a): FITC-5′-Oligo-3′-Biotin 1 µM; anti-FITC-AP antibody 1:12,500; 3-IP 1 mM; Ag⁺ 0.4 mM. (b): Biotin-5′-Oligo-3′-Biotin 1 µM; FITC-5′-Oligo-3′-Biotin 1 µM; anti-FITC-AP antibody 1:12,500; 3-IP 1 mM; Ag⁺ 0.4 mM. Voltammetric scans ((a) and (b)) from −0.02 V to +0.4 V at a scan rate of 50 mV s⁻¹.
References