Functionalized ensembles of nanoelectrodes as affinity biosensors for DNA hybridization detection

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A B S T R A C T

A novel electrochemical biosensor for DNA hybridization detection based on nanoelectrode ensembles (NEEs) is presented. NEEs are prepared by electroless deposition of gold into the pores of a templating track-etched polycarbonate (PC) membrane. The wide surface of the templating membrane surrounding the nanoelectrodes is exploited to bind the capture DNA probes via amide coupling with the carboxylic groups present on the PC surface. The probes are then hybridized with the complementary target labelled with glucose oxidase (GO_x). The occurrence of the hybridization event is detected by adding, to the supporting electrolyte, excess glucose as the substrate and the (ferrocenylmethyl) trimethylammonium cation (FA^+) as suitable redox mediator. In the case of positive hybridization, an electrocatalytic current is detected. In the proposed sensor, the biorecognition event and signal transduction occur in different but neighbouring sites, i.e., the PC surface and the nanoelectrodes, respectively; these sites are separated albeit in close proximity on a nanometer scale. Finally, the possibility to activate the PC surface by treatment with permanganate is demonstrated and the analytical performances of biosensors prepared with $KMnO_4$-treated NEEs and native NEEs are compared and critically evaluated. The proposed biosensor displays high selectivity and sensitivity, with the capability to detect few picomoles of target DNA.

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1. Introduction

The development of electrochemical sensors for DNA detection is widely documented in the recent literature (Kerman et al., 2004; Lucarelli et al., 2004; Hvastkovs and Buttry, 2010; Pedrero et al., 2011). Such sensors are based on the immobilization of an oligonucleotide probe containing the nucleotide sequence of interest onto the surface of the transducer. In this way, due to the presence of the complementary sequence in the sample, the hybridization process takes place directly on the electrode surface, easing the detection.

Different approaches have been employed to enable electrochemical detection of the hybridization event, many of them requiring the use of electrochemical labels such as redox or enzyme labels (Carpini et al., 2004; Levine et al., 2009; Tang et al., 2009) or intercalating redox probes (Girousi and Kinigopoulou, 2010; Wei et al., 2011), or by enabling the label-free detection (Kerman et al., 2003). Recently, the DNA hybridization event was detected by binding the capture DNA onto the surface of complex 3D-structures, such as carbon nanotubes (Abdullin et al., 2007; Li et al., 2003) or

metal nanowires (Lapierre-Devlin et al., 2005). In the latter work, arrayed gold nanowires obtained by partial etching of the polycarbonate surface of nanoelectrode ensembles (NEEs) with oxygen plasma were exploited for the immobilization of the biorecognition layer onto the gold surface of the nanoelectrodes. NEEs are random arrays of nanoelectrodes typically prepared by electroless deposition of gold electrode elements within the pores of a microporous polycarbonate (PC) membrane (Menon and Martin, 1995; Ugo and Moretto, 2007). The diameter and length of the pores in the template determines the geometrical characteristics of the metal nanostructure with radii, usually, of approximately 30 nm and average distance between the nanoelectrodes around 200 nm. For such NEEs the diffusion hemispheres at individual nanoelectrodes overlap and the so-called total overlap diffusion regime, holds. The voltammetric peak current recorded under such conditions is proportional to the overall geometric area of the ensemble (A_{geom}), while the background capacitive current (that is the main component of the noise) is proportional only to the active area (A_{act}). Because of NEEs' geometrical characteristics, the ratio A_{geom}/A_{act} is as high as 10^2–10^3, so that signal/background current ratios are enhanced and detection limits improved 2–3 order of magnitudes (Brunetti et al., 2000; Ugo et al., 1996). Physical (Yu et al., 2003) or chemical (De Leo et al., 2007; Krishnamoorthy and Zoski, 2005) etching allows the controlled removal of the PC which

surrounds the nanoelectrodes so exposing partially or totally (depending on the etching time) the gold nanowires which compose the ensemble. This allows one to increase A_{act} so that larger amounts of biorecognition molecules can be bound on the exposed gold surface. However, such a treatment can cause a not negligible drawback, that is the increase of the double-layer charging current (background noise). It was demonstrated (De Leo et al., 2007) that the etching of NEEs leads indeed to a significant decrease in signal to background current ratio (De Leo et al., 2007), so losing one of the advantages of NEEs.

In order to keep the geometric features intact and the signal/ noise ratio at the maximum, in the present research we developed an alternative approach which is based on exploiting for functionalization/biorecognition purposes the wide polycarbonate surface which surrounds the nanoelectrodes, instead of the nanoelectrodes themselves (Gasparac et al., 2004; Lapierre-Devlin et al., 2005).

Polycarbonate is indeed one of the most used polymeric platforms for the fabrication of bioanalytical sensors for protein or DNA detection (Li et al., 2007; Morais et al., 2006, 2009; Rucker et al., 2005; Wang and Li, 2007); moreover, it is commonly employed as an insulator in the preparation of ensembles and arrays of nanoelectrodes (De Leo et al., 2007; Krishnamoorthy and Zoski, 2005; Menon and Martin, 1995; Moretto et al., 2011). In a recent paper, the polymeric surface of NEEs was employed in our laboratory for the immobilization of antibodies in an electrochemical immunosensor for the detection of the receptor protein HER2 (Mucelli et al., 2008). In that case, the capture antibody trastuzumab was immobilized by direct adsorption (without any pre-treatment) onto the PC surface, which surrounds the nanoelectrodes, leaving the electrodes free for electrochemical transduction. The biosensing capabilities of such NEEs were tested in tumor lysates allowing the detection of HER2 down to 40 pg/μL. In some cases, aspecific adsorption of proteins on the gold nanodisks was observed, limiting the electrochemical signal and, consequently, the detection efficiency. In order to overcome this problem, the possibility to protect the nanoelectrodes with self-assembled monolayers of thiols was studied (Silvestrini et al., 2011). Preliminary attempts performed by us, indicated that for the immobilization of polynucleotides the procedure previously used for proteins, was unsuccessful. This motivated us to perform the present study aimed at developing immobilization procedures specifically devoted at binding DNA probes onto the PC of NEEs.

We demonstrate here that capture oligonucleotide probes can be immobilized on the PC of NEEs by grafting on the −COOH groups naturally present on the PC surface or, eventually, increased in number by a chemical oxidation process. Finally, the hybridization with complementary DNA strand was detected by labeling the target with glucose oxidase (operating in the presence of glucose and a suitable redox mediator) so proving the functionality of the NEE-based hybridization sensor.

2. Experimental

2.1. Materials and apparatus

Potassium permanganate ($KMnO_4$) was purchased from Carlo Erba. Thionin acetate (THA), N-ethyl-N′-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), N-hydroxysulfosuccinimide sodium salt (sulfo-NHS), ethanolamine and glucose oxidase (GO_x) from Aspergillus niger were from Sigma-Aldrich. The salt (ferrocenylmethyl) trimethylammonium hexafluorophosphate ($FA^+PF_6^-$) was prepared by metathesis of the (ferrocenylmethyl) trimethylammonium iodide (Alfa Aesar) with an excess of potassium hexafluorophosphate 99% (Alfa Aesar) (Lombardo and Bieber, 1983). The mixing of the aqueous solutions containing

the two salts, in an ice cooling bath, allowed the formation of a yellow precipitate that was then filtrated, rinsed 5 times with cool water and finally dried in a vacuum oven.

Amino-end DNA (acD1 and acD2) and thiolated DNA (SHD1) were purchased from Sigma-Aldrich with the following sequences:

acD1: [AmC6T]CTTATCGCTTTATGACCGGACC
acD2: [Am5T]TTGTTATACGCC
SHD1: [ThiolC6]GGTCCGGTCATAAAGCGATAAG

Stock solutions of oligonucleotides (100 μM) were prepared in 50 mM Tris, 100 mM NaCl, 1 mM EDTA (TE buffer, pH 7.4) and kept frozen until use. Dithiothreitol (DTT) was from AppliChem. N-2-hydroxyethyl piperazine-N′-2-ethanesulfonic acid (HEPES) ≥ 99.5%, p.a. was purchased from Carl ROTH. The cross-linker sulfosuccinimidyl 4-[N-maleimidomethyl]-cyclohexane-1-carboxylate was purchased from Sigma-Aldrich. Purified water was obtained using Milli-Ro plus Milli-Q (Millipore) water purification system. All other chemicals were reagent grade.

Track-etch polycarbonate (PC) membrane filters were obtained from SPI-pore™ with nominal pore diameter of 30 nm, average pore density of 6×10^8 pores cm^{-2} and coated with polyvinylpyrrolidone (PVP) by the producer.

All electrochemical measurements were carried out at room temperature with a PalmSens potentiostat controlled via personal computer by its own software, using a three-electrode single-compartment cell equipped with a platinum counter electrode and an Ag/AgCl (KCl saturated) reference electrode, to which all reported potential values are referred. Fluorescence measurements were carried out with a Varian, Cary Eclipse Fluorescence Spectrophotometer. NAP™-5 and NAP™-10 columns prepacked with Sephadex™ G-25 DNA Grade were from GE Healthcare. Ultrafiltration devices (Vivaspin6 30000 MWCO) were purchased from VWR. The purification of the DNA-enzyme conjugate was carried out by Fast Protein Liquid Chromatography (FPLC) (GE Healthcare, Äkta Explorer) using a MonoQ 5/50 GL column.

2.2. Synthesis of ssDNA conjugated with GO_x (D1-GO_x)

For the synthesis of ssDNA conjugated with GO_x, a procedure previously described (Fruk et al., 2007) was used. Experimental details are reported in Supplementary information.

2.3. Fabrication, activation and functionalization of NEEs

2.3.1. Template fabrication of NEEs

NEEs were prepared by template gold electroless deposition, using a previously described procedure (De Leo et al., 2007). NEEs were then assembled as previously reported (Ugo and Moretto, 2007). Both procedures are described in Supplementary data.

2.3.2. Characterization and activation of the PC membrane of NEEs

The amount of −COOH groups present on the PC membrane of NEEs was measured by reacting the membrane with thionin acetate (THA) (Ivanov et al., 1996), as follows: a NEE was immersed into 800 μL of a THA solution in ethanol (concentration 0.1 mg mL^{-1}) and shaken overnight at room temperature in dark. The electrode was then removed, washed three times with ethanol and transferred into 800 μL of a 0.01 N HCl solution in a mixture ethanol/water 1:1. After shaking for 1.5 h, the NEE was removed and a fluorescence spectrum of the final acidic solution was recorded (excitation wavelength: 594 nm; emission wavelength: 620 nm).

The number of −COOH groups present on the polymeric surface was increased by adapting the procedure proposed by Papra et al. (1999). Briefly, NEEs were immersed in 0.32 M $KMnO_4$, 0.75 N H_2SO_4 at RT for different times (90 and 150 min), without shaking.

Before the treatment, the insulating tape which surrounds the geometric area of each NEE was protected by a sacrificial layer of adhesive tape (which was removed after the treatment). Subsequently, the electrodes were rinsed with water and 3 M HCl in order to remove any oxidation residues (i.e., manganese oxide) from the polymeric surface, rinsed with water again, then ethanol and, finally, allowed to dry in air.

2.3.3. Functionalization of NEEs

Before proceeding with the immobilization of the oligonucleotide probes, the carboxylic groups were activated by immersion of NEEs into a 0.1 M HEPES buffer solution (pH 7.5) containing 0.5 mM EDC and 0.8 mM sulfo-NHS. The incubation was allowed to proceed for 20 min under shaking (at RT). The addition of the succinimide derivative stabilizes the intermediate ester obtained by reaction between EDC and carboxylate groups (Staros et al., 1986), thus increasing the efficiency of the coupling reaction with the amino-ssDNA (acD1).

The functionalization was completed by transferring the electrodes into a 0.1 M HEPES buffer solution (pH 7.5) containing 400 pmoles of acD1 strands, incubated for 2 h under shaking, at 37 °C. The NEEs were finally rinsed with buffer.

In order to prevent any non-specific adsorption of the target ssD1 conjugated with GO_x (D1-GO_x) on the activated polymeric surface, a blocking treatment was performed. The NEEs were immersed into a 20 mM ethanolamine in 0.01 M PBS solution (pH 7.4). The reaction was allowed to proceed for 30 min at RT, under shaking. Ethanolamine is used to deactivate the remaining NHS-activated carboxylic groups (Park and Abbott, 2008) onto the polymeric membrane without interfering with biological macromolecules present on the surface of the NEE (Liberelle et al., 2010).

The hybridization reaction was carried out by spotting 10 μL of a 0.01 M PBS solution (pH 7.4) containing different amounts of D1-GO_x (from 2.5 to 20 pmoles) onto the capture DNA containing surfaces and incubating for 2 h at RT. Resulting DNA-modified NEEs were rinsed five times in 0.01 M PBS over 10 min, before being used for the electrochemical detection in the suitable electrolyte solution.

3. Results and discussion

3.1. Characterization and functionalization of NEEs

At first, the PC surface of a NEE was characterized by spectrochemical titration with thionin acetate (see experimental) in order to estimate the amount of naturally occurring surface carboxylic groups available for the probe immobilization. The thionin procedure was used previously for the quantitative determination of carboxylic groups on activated polymers (Geismann and Ulbricht, 2005; Médard et al., 2001, 2002); it is based on the formation of ion pairs between the cationic dye and carboxylate moieties. A calibration curve was first obtained and subsequently employed for the quantification of the carboxylic groups. The obtained concentration of −COOH groups determined on the polymeric surface of the NEE was about 9.7×10^{-10} mol cm^{-2}.

These terminal functional groups of the polymeric chains present on the surface were then exploited for the immobilization of single stranded DNA (acD1) using EDC/NHS strategy (Staros et al., 1986) (Scheme 1a).

To enable the detection of the DNA hybridization event, thiolated complementary DNA (SHD1) was modified with glucose oxidase (GO_x) using sulfoSMCC methodology (Fruk et al., 2007) and subsequently hybridized to the capture acD1 immobilized on the PC (Scheme 1b). Glucose oxidase was chosen as the enzymatic label since it is readily available; moreover, the modification with single-stranded DNA is simple and it enables the amplification of the redox probe signal (Cass et al., 1984).

Electrochemical characterization of functionalized NEEs was performed in a deoxygenated PBS solution (10 mM, pH=7.4) containing 0.1 mM FA$^+$ as the redox mediator. It should be noted that the solution was purged with nitrogen in order to prevent the eventual inhibition of GO_x enzymatic activity by H_2O_2, which is eventually produced by the competing reaction between the enzyme and the substrate, in presence of oxygen acting as an electron acceptor (Kleppe, 1966).

The full line voltammogram in Fig. 1a shows the CV behavior of FA$^+$ recorded using acD1 modified NEE, spotted with 20 pmoles of D1-GO_x. The voltammetric pattern is reversible and diffusion controlled ($\Delta E_p = 0.067$ V, $E_{1/2} = (E_{pa} + E_{pc})/2 = 0.42$ V, $I_{pc}/I_{pa} = 1$). In particular, the evidence that I_p scales linearly with v1/2 (data not shown), indicates that the functionalization of the NEE with the DNA probe does not interfere with the total overlap diffusive regime (Menon and Martin, 1995) which characterizes the FA$^+$ voltammetric behavior at non-functionalized NEEs (Silvestrini et al., 2011). Note that the CV pattern recorded with a bare NEE (Fig. 1a, dotted line) overlap with the one recorded at the functionalized NEE, in the absence of the label substrate (Fig. 1a, full line). In the presence of 50 mM glucose (Fig. 1a, dashed line), the voltammetric pattern, at the functionalized NEE, changes dramatically: the oxidation peak increases tending to a sigmoidal shape while the reduction peak disappears. The shape of the voltammogram is typical for an electrocatalytic process: in the presence of both enzyme label and the enzymatic substrate, the mediator is reduced chemically at the electrode/solution interface to be oxidized again directly at the electrode surface. Therefore, the above described voltammetric evidences indicate that the functionalization with the capture probe and the subsequent hybridization with the GO_x labeled target on the NEE are successful. Moreover, it is demonstrated that GO_x acts as a sensitive and efficient label, suitable to detect the hybridization event at the NEE. Fig. 1b shows that a well detectable electrocatalytic current increase is observed also when the target amount is lowered to 2.5 picomoles.

Data reported in Fig. 2 show the change in electrocatalytic current as a function of the amount of target spotted on the sensor. A linear trend is observed with a sensitivity of 25 nA/pmoles. Repeated measurements performed on three different
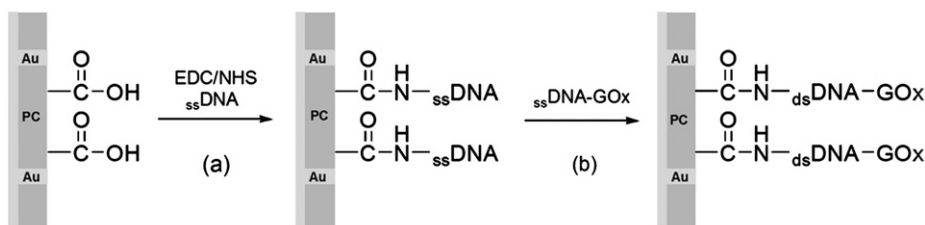
Scheme 1. Design of DNA hybridization sensor based on NEE assembly: (a) activation of −COOH groups of the PC surface and immobilization of the capture complementary acD1 onto the activated carboxylic functionalities and (b) hybridization of DNA-GO_x conjugate onto modified PC surface.
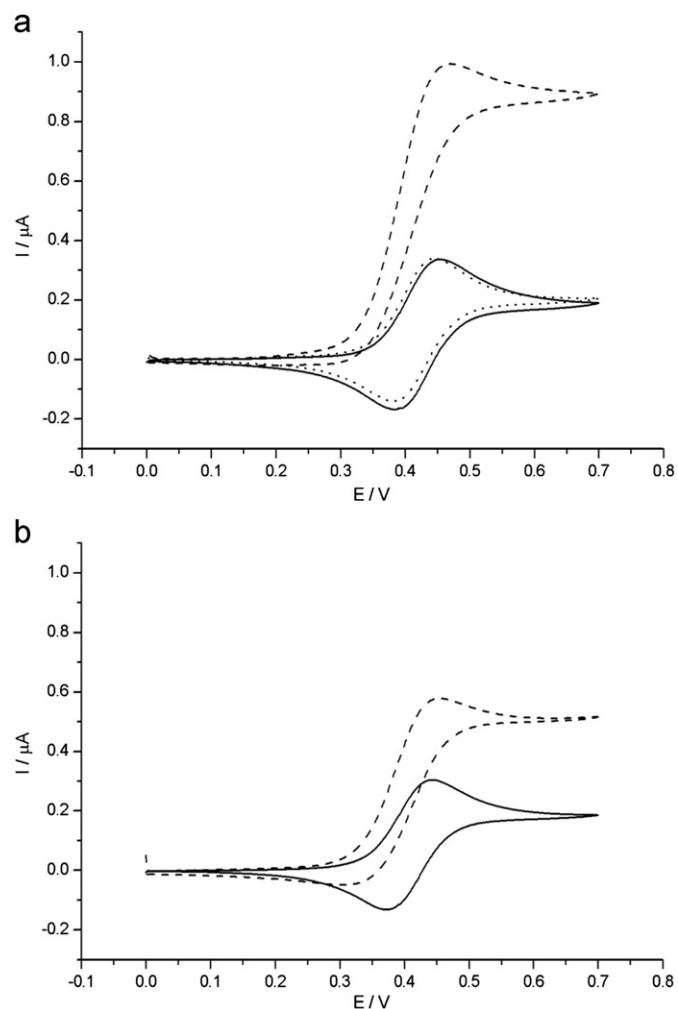
a

b

Fig. 1. Cyclic voltammograms recorded with NEEs functionalized with acD1 and treated with either (a) 20 pmoles or (b) 2.5 pmoles D1-GO$_x$ in a 10 mM PBS solution (pH=7.4) containing 0.1 mM FA$^+$PF$_6^-$ before (full line) and after (dashed line) the addition of 50 mM glucose. Control in which bare NEE is used is represented by the dotted line (a). Scan rate 5 mV s^{-1}.

NEEs indicated that the standard deviation (σ) measured on ΔI_{cat} (where $\Delta I_{cat} = I_{cat}$ in the presence of glucose—I_{peak} in the absence of glucose) is 1 nA. This value indicates a very good precision for the detection; interestingly, this σ value is 10 times smaller than the standard deviation (namely, 10 nA) measured on three different NEEs, functionalized as above described, for the FA$^+$ signal alone. This suggests that the electrocatalytic current increase (which in our conditions is proportional to the amount of GO$_x$ label immobilized on the PC surface of the NEE) is less sensitive to the variability in the NEE fabrication procedure than the peak current measured for redox probes under pure diffusion control (Pereira et al., 2006). The minimum detectable amount of target DNA, based on the 3 σ criterion, was estimated 0.1 pmoles.

Fig. 3 shows the CVs recorded with non-functionalized NEEs when mediator (0.1 mM), substrate (50 mM) and 5 nM (20 pmoles) unmodified GO$_x$ (Fig. 3a, 0.42 μA) or 5 nM (20 pmoles) D1-GO$_x$ (Fig. 3b, 0.55 μA) are present in the solution. Comparison with Fig. 1a indicates that the anodic current value measured with the DNA functionalized NEE in the presence of the glucose is significantly higher than the current values detected with the bare NEE.

It is worth stressing that, in order to achieve a comparable catalytic current value as in Fig. 1a, the amount of GO$_x$ in solution, in experiments like those in Fig. 3a, must be increased up to
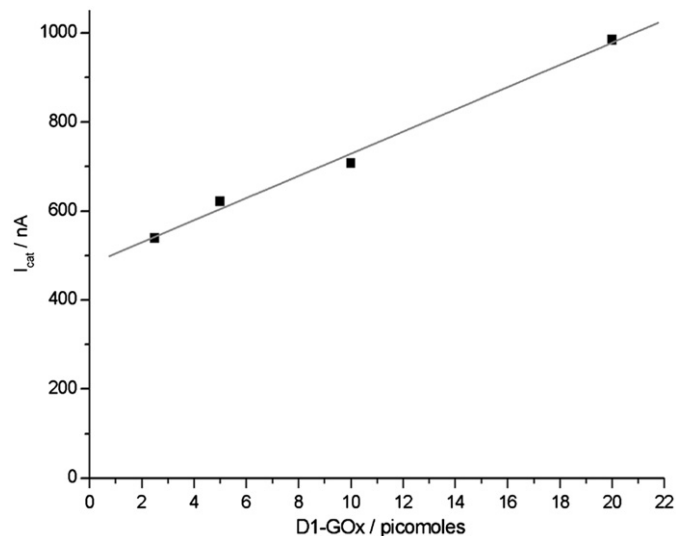

Fig. 2. I_{cat} values recorded with NEEs functionalized with acD1 and treated with different amounts of D1-GO$_x$ (2.5; 5; 10; 20 pmoles), in a 10 mM PBS solution (pH=7.4) containing 0.1 mM FA$^+$PF$_6^-$. Scan rate 5 mV s^{-1}.
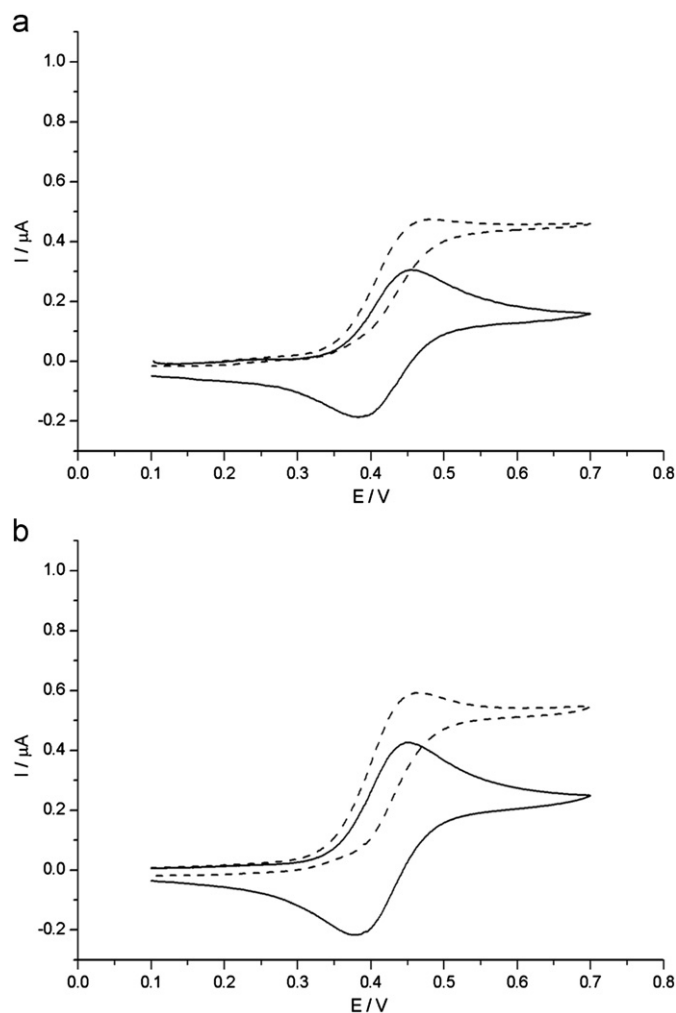
a

b

Fig. 3. Cyclic voltammograms recorded with a bare NEE in a 10 mM PBS solution (pH=7.4) containing 0.1 mM FA$^+$PF$_6^-$ and 50 mM glucose before (full line) and after (dashed line) the addition to the electrolyte solution of (a) 20 pmoles GO$_x$ and (b) 20 pmoles D1-GO$_x$. Scan rate 5 mV s^{-1}.

200 pmoles. These results confirm the high capture efficiency of D1-GO$_x$ by the acD1 functionalized NEE. Control experiments using non-complementary capture DNA (acD2) and D1-GO$_x$ did not show any significant electrochemical activity (Fig. S1, Supplementary data) and confirmed that non-specific adsorption of the labeled target is negligible.

These results indicate a good detection capability for the functionalized NEEs with detection limits below the picomoles.

3.2. Effect of the chemical activation of NEEs

In order to further increase the detection performances demonstrated above, we tested the possibility of increasing the surface concentration of −COOH groups on the PC surface and, consequently, the amount of probe which can be bound, by treatment with KMNO$_4$ as described in earlier reports (Papra et al., 1999; Thorp et al., 1999). The number of generated carboxylic groups was again determined by fluorescence labeling using thionin acetate (THA). The fluorescence intensities of the acidic solutions containing THA and the relative calculated amounts of −COOH per cm^2, obtained after 90 and 150 min of oxidative treatment of the NEEs with KMNO$_4$, are listed in Table 1 and compared with those measured for the non-activated NEE.

These data show a significant increase in surface −COOH concentration upon KMNO$_4$ activation; for instance, a 150 min treatment leads to a three-fold increase in the surface concentration of carboxylic groups.

These activated electrodes were then functionalized with the acD1 capture probe and hybridized with the D1-GO$_x$ target using the same procedure as described for non activated NEEs. Fig. 4 shows the voltammetric responses recorded in FA$^+$ solution with activated NEE (90 min) to which D1-GO$_x$ was immobilized, before and after the addition of the excess glucose (full and dashed lines, respectively). The voltammetric signal of the mediator recorded before the glucose addition differs from the one reported in Fig. 1a, mainly for the larger capacitive current which is evident between 0.0 and 0.2 V, i.e., before the rising of the Faradaic signal (CV peaks). After adding excess glucose, the Faradaic anodic current increases up to 1.24 μA and the peak tends to a sigmoidal shape, while the associated cathodic peak (return scan) disappears. All these features are comparable to those observed for the DNA-GO$_x$ on non-activated NEE, apart the significant increase in the double-layer (background) charging current. Note that the ΔI_{cat} value (difference in anodic peak current with and without glucose) is now slightly higher being 0.8 μA at the KMNO$_4$-activated (Fig. 4) and 0.64 μA at non-activated (Fig. 1a). NEE. Control experiments (data not shown) again showed that there is no significant non-specific adsorption of the DNA-GO$_x$ target at the activated NEE.

The above results indicate that both the functionalization with the capture DNA probe and the hybridization with the GO$_x$ labeled target can be performed successfully using KMNO$_4$ activated NEEs. Furthermore, the amount of DNA captured by the activated electrode is slightly higher than in the case when there is no prior treatment, as indicated by the 15–20% Faradaic current increase for the activated vs. non-activated NEE. However, the activation of the NEE with KMNO$_4$ causes an undesired increase in the background current.

Table 1
Concentration of −COOH groups measured by the thionin acetate method, after activation of the PC surface with the KMNO$_4$ for the indicated time.

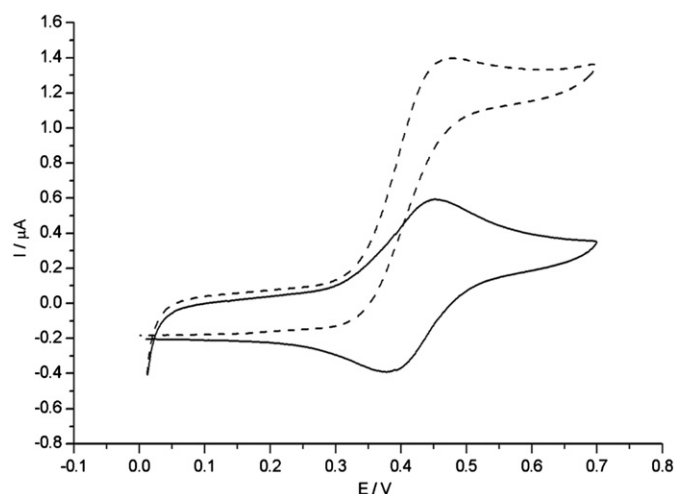| Time of treatment with KMNO$_4$ (min)	Intensity (a.u.)	Concentration of COOH groups (mol cm^{-2})
0	7.7	$\sim 9.7 \times 10^{-10}$
90	21.1	$\sim 2.7 \times 10^{-9}$
150	26.9	$\sim 3.4 \times 10^{-9}$

Fig. 4. Cyclic voltammograms recorded with a NEE activated with KMNO$_4$, functionalized with acD1 and treated with 20 pmoles of functionalized D1-GO$_x$. Measurements were performed in a 10 mM PBS solution (pH=7.4) containing 0.1 mM FA$^+$PF$_6^-$ before (full line) and after (dashed line) the addition of 50 mM glucose. Scan rate 5 mV s^{-1}.

Table 2
Active area values of NEEs after treatment with KMNO$_4$, as a function of the oxidation time.

Time of treatment with KMNO$_4$ (min)	A_{act} (cm^2)
0	3.2×10^{-3}
90	3.7×10^{-2}
150	0.3

In order to investigate the latter effect, a series of background CVs were recorded at bare NEEs in pure supporting electrolyte (0.01 M KNO$_3$), before and after the chemical activation (Fig. S2, Supplementary data); A_{act} values were obtained by relevant capacitive current values (Menon and Martin, 1995); relevant results are summarized in Table 2.

The A_{act} values scale with the time length of the KMNO$_4$ treatment. This can be due either to the partial etching of the outer layer of the polymer and/or the loss in sealing between the Au nanofibers and the surrounding membrane. Both these conditions could cause a partial exposure of the fibers, increasing the area of the metal surface in contact with the solution (De Leo et al., 2007). SEM observations (not shown) confirmed this hypothesis, showing that after, e.g., 90 min oxidation with KMNO$_4$, the tips of the nanoelectrodes emerged from the PC substrate for approximately 80–100 nm. Moreover, some slits were formed at the junction between the gold nanofibers and PC, so producing a final morphology similar to that of the 3D-NEEs previously obtained by chemical etching of NEEs with ethanol-dichloromethane (De Leo et al., 2007).

From the data obtained we can conclude that the activation treatment increases the sensitivity of about 20%; however, this procedure also causes an increase in the background current and requires a more complex functionalization procedure.

4. Conclusions

We have, for the first time, shown that the polymer surface of nanoelectrode ensembles can be bio-functionalized with DNA, by utilizing the amide coupling strategy to immobilize single-stranded

DNA onto the PC, instead that onto the gold electrode surface (Gasparac et al., 2004). Electrochemical detection of hybridization with complementary strand containing a reporter enzyme GO_x and with the help of a redox mediator, results effective and sensitive allowing one to detect few pmoles of target DNA. The oxidation of the polymer surface by permanganate can be used to increase the number of $-COOH$ groups present on the PC; however, this reflects in an increase in the capacitive current, due to the partial etching of the PC. Detection capabilities of non-oxidized NEEs are surely attractive, however with the notation that when larger amounts of target DNA need to be captured, the activation of PC can be used. Further studies on the long term stability for the sensor as well as on the possibility to improve detection capabilities by using pulsed voltammetric techniques are in progress.

Acknowledgements

Appendix A. Supplementary information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bios.2012.07.041.

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