Enhanced Electrochemical Detection of DNA Hybridization Based on Electrode-Surface Modification

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We report an improved electrochemistry-based sequence-specific detection technique by modifying the electrode surface using polyelectrolytes or utilizing the electrode whose surface exhibits the lowest background signal. The objective of the present work is to develop DNA-detection platforms that offer high sensitivity and selectivity in transducing the hybridization event and are compatible with the microfabrication process. Gold nanoparticle labels, coupled with signal amplification by the silver-enhancement technique, would be a suitable choice of detection scheme. However, common electrodes used in microfabrication, such as gold, are susceptible to silver depositions, making the extraction of the analytical signal (silver on a gold nanoparticle) from the background signal (silver on a gold substrate) impossible. In this study, the background silver deposition on the gold electrode is significantly reduced by modifying its surface with polyelectrolyte multilayer films of poly(allylamine hydrochloride) and poly(styrenesulfonate). Moreover, indium tin oxide (ITO), a conductive material that is compatible with microfabrication, is found to exhibit low silver-enhancing properties. With the low background signal achieved by electrode-surface modification and selection, sensitive (high signal-to-noise ratio) electrochemical detection of the hybridization event using the silver-enhanced gold nanoparticle approach is demonstrated. The gold and ITO-detection platforms described here are readily applicable to miniaturized bioanalytical microdevices for integrated and point-of-care viral and bacterial infections diagnostics.

Introduction

The development of an integrated DNA analytical microdevice holds great potential for the decentralized screening of infectious diseases. One of the challenges ahead in the realization of a hand-held DNA analyzer is the ability to perform on-chip sequence-specific detection. In this regard, routine optical detection methods[1] used in diagnosis laboratories have to be replaced with other miniaturizable techniques. The electrochemical method, attributed to its inherent advantage of miniaturization, is promising for rapid and low-cost DNA analysis in microchip setting.[2] The most widely studied electrochemical DNA sensors are based on electroactive DNA intercalators, which include metal complexes,[3−6] anthracycline antibiotics,[7] bisbenzimide dyes,[8,9] and ferrocene derivatives.[10] Nevertheless, the intercalator-based assays suffer the drawback of being not highly sensitive and selective. For a sensitive detection scheme, the signal-to-noise (S/N) ratio should be as high as possible. Other transduction schemes that label the target DNA with redox-active groups, including enzymes[11−13] and ferrocene,[14] have been devised to improve the S/N ratio.

Recently, Mirkin and co-workers[15] demonstrated the use of the gold nanoparticle probe for a highly sensitive scanometric detection of the hybridization process. The high sensitivity of this approach is ascribable to signal amplification by silver enhancement.[16] In this process, silver metals are catalytically deposited onto the gold nanoparticle surface (but not onto the glass support), resulting in an extraordinarily high S/N ratio. This initial study also discovered the exceptionally sharp melting curve of a DNA hybrid with gold nanoparticle labels, which translates into high-recognition selectivity for single base mismatch differentiation. To adopt the metal nanoparticle-based detection scheme in a DNA biochip, the electrochemical detection of the nanoparticle label is highly desirable. Several groups have reported the electrochemical detection of target sequences with gold[17,18] or silver[19] nanoparticle labels. The amount of bound nanoparticle labels was determined by the acidic dissolution of the

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nanoparticle, followed by a stripping analysis of the dissolved metal ions. Wang et al.[20] and Fang et al.[21] have also successfully incorporated the silver-enhancement technique with the gold nanoparticle labels for enhanced electrochemical hybridization detections. In the latter case, the silver deposits on the gold nanoparticle label were measured directly by its oxidation current response during voltammetric scans.

All previous studies on the silver and gold nanoparticle-based electrochemical hybridization detection utilize supports of polystyrene microwells,[17] magnetic beads,[18,20] and carbon electrodes,[19,21] which are difficult to implement on-chip. Aiming at an integrated microchip, the detection platform should ideally be microfabricated electrodes. Standard electrode surfaces such as gold and platinum, however, are vulnerable for significant silver depositions, which create difficulty in distinguishing whether the electrochemical signal of the silver-dissolution current is from the DNA-bound nanoparticle tag or background electrode surface. In this work, we demonstrate that the background deposition of silver can be curtailed by creating a positively charged electrode surface using the electrostatic self-assembly of polyelectrolytes. As a result, the S/N ratio, as well as sensitivity of the detection technology, is significantly improved. The effect of the layer-by-layer adsorption of the polyelectrolyte [poly(allylamine hydrochloride) (PAH) and poly(styrenesulfonate) (PSS)] on the background silver deposition is investigated. Moreover, the electrode surface made of indium tin oxide (ITO), which is a material inherently immune to the silver deposition and compatible with the microfabrication, will be explored. Finally, the capability of the polyelectrolyte-modified (PEM) gold and the ITO electrodes to detect specific DNA sequences using the silver-enhanced gold nanoparticle label is demonstrated by synthetic oligonucleotides.

2. Experimental Section

Reagents. Four oligonucleotides were purchased from Synthetic Genetics (San Diego, CA). Three of them have the base sequence 5′-CAC AAA ACG GGG GCG G-3′, including a probe for the PEM gold electrode (modified with a biotin group at the 5′ end), probe for the ITO electrode (modified with a mercaptohexyl group at the 5′ end), and positive control for the ITO experiment (modified with a mercaptohexyl group at the 5′ end and a biotin group at the 3′ end). Note that the probe for the PEM gold electrode is also used as a negative control (noncomplementary to the probe). The other one has the base sequence 5′-CCG CCC CCG TTT TGT G-3′, namely, target (complementary to the probe, modified with a biotin group at the 5′ end).

Dithiothreitol (DTT), hydrochloric acid (38%), avidin (from egg white), *N*-(2-hydroxyethyl)piperazine-*N*′-(2-ethanesulfonic acid) (HEPES), streptavidin/colloidal gold (10 nm), and a silver-enhancement kit were obtained from Sigma (St. Louis, MO). Sodium acetate, sodium chloride, disodium hydrogen phosphate, sodium dihydrogen phosphate, hydrogen peroxide (35%), sodium hydroxide, trisodium citrate dihydrate, sodium nitrate, and potassium nitrate were of the RdH brand (Sigma-Aldrich, Germany). Ethanol (absolute), nitric acid (70%), sulfuric acid (98%), 11-mercaptoundecanoic acid (MUDA), PAH (M_w ca. 70 000), poly(sodium 4-styrenesulfonate) (M_w ca. 70 000), manganese(II) chloride tetrahydrate, Alconox, propan-2-ol, and 3-mercaptopropionic acid (MPA) were purchased from Aldrich (Milwaukee, WI). All chemicals were of analytical reagent grade and were used as received. All solutions were prepared with ultrapure water from a Millipore Milli-Q system.

Instrumentation. Linear sweep voltammetry was performed using an Autolab PGSTAT 30 potentiostat/galvanostat (Eco Chemie, The Netherlands) controlled by the General Purpose Electrochemical System (GPES 4.8) software (Eco Chemie). The electrochemical measurements were carried out in a 50-mL cell using a Ag/AgCl reference electrode (immersed in a 3 M NaCl filling solution saturated with AgCl; EG & G, Princeton Applied Research, Oak Ridge, TN) and a platinum wire counter electrode. The working electrode was either gold (area of 1.37 cm^2; Maxtek, Torrance, CA) or ITO (dimensions of 1 × 1 cm, kindly provided by Samsung, Korea). The DNA concentration was determined with a Genequant RNA/DNA calculator (Amersham Biosciences, Piscataway, NJ).

Procedures. *Preparation of the Thiol-Modified Probe Solutions.* Thiol-modified DNA tends to undergo oxidative dimerization to form disulfide, resulting in a reduced probe-immobilization efficiency and variable surface probe density for different batches of experiments. To reduce dimerization, the thiol-modified probes (the probe and positive control for the ITO experiments) were treated with DTT prior to their immobilization. The probe/DTT solution (the final concentration of DTT was 0.1 M) was incubated at room temperature for 30 min, followed by the removal of DTT using a standard ethanol precipitation procedure.[22] Briefly, 1 vol of the probe/DTT solution was mixed thoroughly with 0.1 vol of 3 M sodium acetate (pH 5.6) and 3 vol of ice-cold absolute ethanol, kept at −80 °C for 30 min and centrifuged at 14 000 g for 15 min. The DNA pellet obtained was washed with 1 mL of 95% ethanol, centrifuged again, dried, and then resuspended in water. The DNA concentration in this solution was determined and adjusted to 25 μM (the final salt composition was 500 mM NaCl/25 mM sodium phosphate, pH 7.0) as the probe immobilization solution.

Gold-Electrode Pretreatment and Modification. The same set of gold electrodes was reused throughout this work. The electrodes were sequentially cleaned with HNO_3 and H_2SO_4/H_2O_2 (volume ratio of 7:3) solutions in a sonic bath for 5 and 10 min, respectively, followed by a thorough rinsing with water and drying with nitrogen. The cleaned electrodes were immersed into an ethanolic solution of MUDA (2 mM) for 48 h at room temperature, again rinsed with water, and dried. The layer-by-layer deposition of the polyelectrolytes by alternating the adsorption of the polycations (PAH) and polyanions (PSS) was performed as follows[23,24] with water rinsing and nitrogen drying after every adsorption step. The MUDA-modified electrodes were soaked in a 3 mg/mL PAH solution (2 M NaCl, pH 8.0, adjusted by NaOH) for 20 min. Similarly, the PAH-coated electrodes were exposed to a 3 mg/mL PSS solution (1 M $MnCl_2$, 0.01 M HCl, pH ∼2.0) for 20 min. For the hybridization-based DNA-detection experiments, a total of six layers [$(PAH/PSS)_3$] were formed on the gold-electrode surface. Then, avidin was adsorbed onto the outermost PSS layer by applying a 0.1 mg/mL solution (0.2 M NaCl, 0.05 M HEPES, pH 7.5) for 1 h. Excess avidin was washed off with large quantities of water.

ITO-Electrode Pretreatment and Modification. The ITO electrodes were cleaned according to the procedure reported elsewhere.[25] Briefly, they were sequentially sonicated in an Alconox solution (8 g of Alconox/1 L of water), propan-2-ol, and twice in water, each sonication lasting for 15 min. Subsequently, the ITO electrodes were modified with a self-assembled monolayer (SAM) of MPA by immersion in an aqueous 5 mM solution for 24 h. The electrodes were flushed with copious amounts of water to remove any physically adsorbed MPA.

Probe Immobilization. The probe was immobilized onto the PEM gold electrode via an avidin−biotin interaction. The biotinylated probe was immobilized onto the avidin-coated substrate by exposure to a 5 μM solution (0.2 M NaCl, 0.05 M HEPES, pH 7.5) for 1 h. Probe immobilization onto the ITO electrode was achieved through disulfide linkage. After the DTT treatment, 5 μL of the probe-immobilization solution (25 μM) was spotted onto the MPA-modified ITO-electrode surface. The

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coupling reaction was allowed to proceed for 12 h in a humidity chamber. Excess probe was removed by water rinsing.

DNA Hybridization. Oligonucleotide (the target or negative control) solutions of different concentrations (10 nM−1 μM) were prepared in a 0.3 M NaCl/30 mM sodium citrate solution (SSC; 2 × SSC, pH 7.0). For the hybridization experiments with the gold and ITO electrodes, 200 and 5 μL of the samples were applied to the probe-modified electrode surfaces, respectively. After 60 min of incubation at room temperature, the electrodes were rinsed thoroughly with 2 × SSC buffer to remove nonspecifically bound species.

Gold Nanoparticle Label Binding and Silver Enhancement. The gold nanoparticle label was bound to the hybridized target or positive control by incubating the streptavidin/gold solution on the electrode surface at room temperature for 1 h. The unbound gold nanoparticle label was removed by rinsing with phosphate-buffered nitrate solution (PBN, 0.3 M $NaNO_3$/10 mM sodium phosphate, pH 7.0). Then, silver deposition was performed in accordance with the manufacturer's instructions. The duration of the silver enhancement was typically set to 8 min, unless otherwise stated. After that, the electrode was rinsed with water to remove the silver-enhancement solution.

Electrochemical Measurements. The amount of silver deposited onto the gold nanoparticle label was determined by measuring the oxidative silver-dissolution current during the linear sweep voltammetric (LSV) scan. The measurements were conducted at a scan rate of 100 mV/s with 0.1 M KNO_3 as the supporting electrolyte. Note that all measurements were taken at room temperature.

3. Results and Discussion

The exceptionally high sensitivity and selectivity of the metal nanoparticle labels for hybridization detection have been revealed using optical,[15] mass-sensitive,[26] electrical,[27] and electrochemical[17−21] methods. Among these, the electrochemical technique is well-suited for integrated microfluidic bioanalytical applications. However, previous studies of metal nanoparticle-based hybridization detection are performed on solid supports not amenable to miniaturization. In the following sections, we demonstrate the capability of employing the gold and ITO electrodes, which are common materials in the microfabrication, for sensitive hybridization detection with the silver-enhanced gold nanoparticle approach.

Gold-Electrode Modification and Hybridization Detection. One immediate problem associated with the use of the gold electrode for the silver-enhanced metal nanoparticle approach is the serious background staining. When the gold electrode is exposed to the silver-enhancement solution, catalytic silver deposition occurs at the electrode surface and turns the color from golden to dark gray, as shown in the inset of Figure 1. The silver deposition is confirmed by the LSV scan of the gold electrode (Figure 1), which exhibits an oxidation current peak at 0.65 V (with respect to the Ag/AgCl reference electrode). This high background signal (∼10 mA) has a deleterious effect on the S/N ratio, thereby making the direct use of the gold electrode for silver-enhancement-based assay impossible.

One way to minimize the background silver deposition is by positively charging the electrode surface. With this, the silver ions are retarded from approaching the gold surface and, thus, preventing silver deposition on the electrode surface. Polyelectrolyte multilayer films have long been utilized for well-controlled surface-charge modification.[28] Figure 2 illustrates a schematic representation of the structure of the polyelectrolyte films

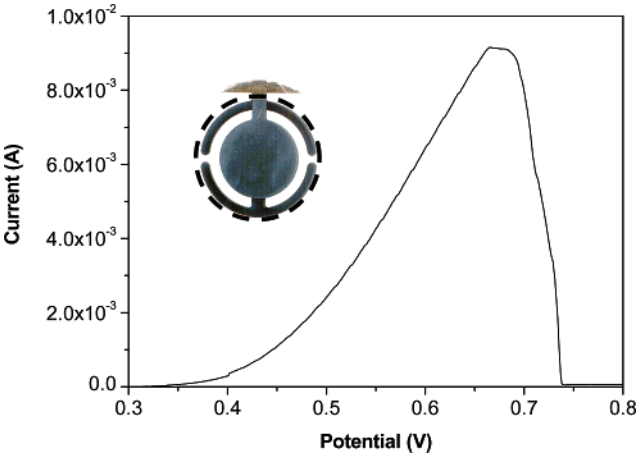

Figure 1. LSV scan of the gold electrode (area of 1.37 cm^2) subjected to silver enhancement for 5 min. Inset: image of the gold electrode that appeared dark gray after the silver enhancement. The dashed black circle marked the area that was exposed to the silver-enhancement solution.

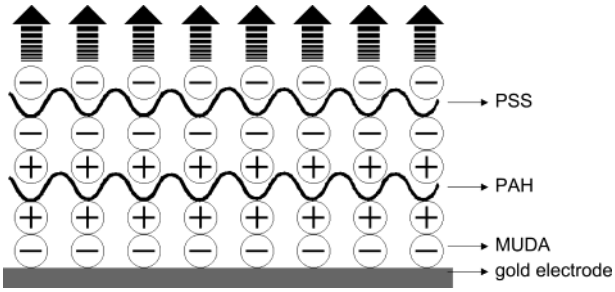

Figure 2. Illustration of the structure of electrostatic self-assembled polyelectrolyte multilayer films by the alternating adsorption of polycations (PAH) and polyanions (PSS).

adsorbed onto the gold-electrode surface. In principle, the gold template has to be equipped with a minimal amount of charge before the polyelectrolyte adsorption can take place. In our case, the gold surface is first self-assembled with a monolayer of MUDA, creating a negatively charged template. Then, PAH is electrostatically adsorbed onto the MUDA-coated template, resulting in a positively charged surface. Similarly, negatively charged PSS can be deposited on top of the PAH layer. The alternating adsorption of PAH and PSS continues until the desired number of layers is reached. The effect of the number of PAH layers on the background silver-deposition signal is presented in Figure 3. The oxidation current of the silver deposits decreases as the number of PAH layers increases. There is a 20-fold reduction in the silver oxidation charge (area under the LSV curve with background-current correction) for the gold electrode modified with three layers of PAH when compared to that for the bare electrode. This significant decrease in the background staining can be explained not only by the stronger repulsive force at the outermost surface but also by the stronger diffusion barrier for the silver ions as the film grows. Further reduction in the background signal can be achieved by increasing the number of polyelectrolyte layers.

Figure 4 is a schematic that depicts how the PEM gold electrode can be applied for the DNA-hybridization detection with the silver-enhanced nanoparticle approach. The gold electrodes for the hybridization experiments are coated with six polyelectrolyte layers [$(PAH/PSS)_3$] for which the background is sufficiently low for a sensitive

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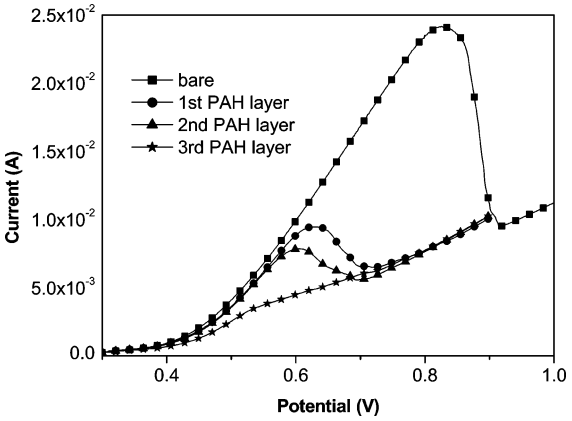

Figure 3. LSV scan of silver deposited on the gold electrodes modified with different numbers of PAH layers. The PEM gold electrodes were exposed to the silver-enhancement solution for 8 min.

detection using the silver-enhanced gold nanoparticle approach. The outermost negatively charged PSS layer is used to bind the positively charged avidin, which is similar to the interaction between PSS and PAH. The immobilization of the DNA probe is accomplished through avidin−biotin binding. The gold electrode is now prepared for hybridization with DNA analytes. The gold nanoparticle hybridization indicator is bound to the hybridized target by the interaction between the biotin group of the target and the streptavidin group of the gold nanoparticle. An alternative linkage such as the direct chemisorption of the 5′ thiol-modified sensing probe on the gold nanoparticle is also possible.[15] Subsequent silver depositions on the gold nanoparticle result in a remarkable signal amplification to such an extent that the visual determination of the hybridization event is permitted. The significance of the current approach rests on the ability to take advantage of the silver deposits as an electrochemical signal to transduce the hybridization event but does not rest on the optical characteristics.

Figure 5 shows that the LSV response of the probe-modified gold electrode exposed to the target sequence is higher than that exposed to the negative control. This indicates the capability of the PEM gold electrode to electrochemically transduce the hybridization event using the gold nanoparticle label coupled with signal amplification by silver enhancement. It should be noted that the silver oxidation peak of the probe-only PEM gold-electrode surface is almost half of the one with the target. This background signal is caused by silver deposition onto the probe through Ag^+/Na^+ ion exchange along the polyanionic DNA backbone and through the formation of complexes between the silver ions and the DNA bases.[29] In addition, another source of the background comes from the silver particulates formed in solution (nonspecific self-catalysis) and precipitated onto the electrode surface. These silver depositions can be significantly reduced by adding a sodium thiosulfate fixer solution to the silver-enhancement solutions[20] and using new silver-enhancement solutions every 2−3 min,[27] respectively.

ITO-Electrode Modification and Hybridization Detection. Common electrodes such as carbon, gold, and platinum suffer from the serious setback of background staining. ITO, a conductive material, on the other hand, has a low silver-enhancing property. When an ITO electrode is exposed to the silver-enhancement solution for 5 min (similar to the experiment conducted at the bare gold electrode, Figure 1), the electrode remains transparent, without any gray color development, as manifested in the inset of Figure 6. The amount of the silver metal deposited onto the ITO electrode (area close to that of the gold electrode) is again measured by the LSV technique. The total charge of the ITO electrode is 40 times less than that of the bare gold electrode (compare Figure 6 with Figure 1), demonstrating the superior performance of the ITO electrode for the electrochemical transduction of the silver-enhanced gold nanoparticle.

To immobilize the DNA probe, the ITO electrode is first modified with a SAM. The SAM is a heterobifunctional linker that binds to the ITO surface at one end of the molecule and the probe at the other end. Here, the SAM of MPA is formed at the electrode surface based on the known affinity of the carboxylic group toward the ITO surface.[30,31] The pendant thiol group of the MPA monolayer is then coupled with the 5′ thiol-modified probe via the disulfide bond. This probe-modified electrode surface can now be used for the hybridization-based detection of DNA.

A series of four ITO electrodes are included to study the electrochemical sequence-specific detection of DNA with the silver-enhanced gold nanoparticle approach. Panels A−C of Figure 7 illustrate the ITO electrodes modified with the probe, whereas that in panel D was modified with the positive control. As a result of the fact that the positive control, which has an identical sequence and the 5′ thiol group as the probe, has a biotin group at its 3′ end, the ITO surface should always turn gray after the silver enhancement. Therefore, the inclusion of panel D ensures proper immobilization of the thiolated probe on the MPA-modified ITO surface. If nonthiolated DNA (i.e., the positive control without the 5′ thiol group) is used, the gray color development does not occur (image not shown). To evaluate the capability of the probe-modified ITO electrode to differentiate the complementary DNA analyte from noncomplementary ones, hybridizations are carried out with the target (panel B) and negative control (panel C). The gray color observed in panel B is intermediate between that in panels A (the probe surface) and D, implying that not all probe sites are accessible to hybridization with the target. On the other hand, the color intensity of panel C is similar to that of panel A, indicating that the negative control does not bind nonspecifically to the probe-modified surface. Again, this apparent visual difference in color can be quantified electrochemically by oxidizing the silver metal deposited on the gold nanoparticle label (given in Figure 7). The oxidation current responses of the four ITO electrodes are consistent with their corresponding color intensities.

The LSV scans of the probe-modified ITO electrodes hybridized with different concentrations of the target sequence are given in Figure 8. It can be seen that the silver-oxidation current peak is proportional to the concentration of the target DNA analyte. A correlation curve between the target concentration (over the range of 10 nM−10 μM) and total charge of the oxidation of the silver deposits is given in the inset of Figure 8. Under the existing experimental conditions, a dynamic range from 10−100 nM for the target DNA is attained. Nevertheless, the data points shown in the curve (10, 50, and 100 nM) do not lie well on a straight line. To achieve a highly linear response of the signal versus target concentration, more data points within the dynamic range should be obtained

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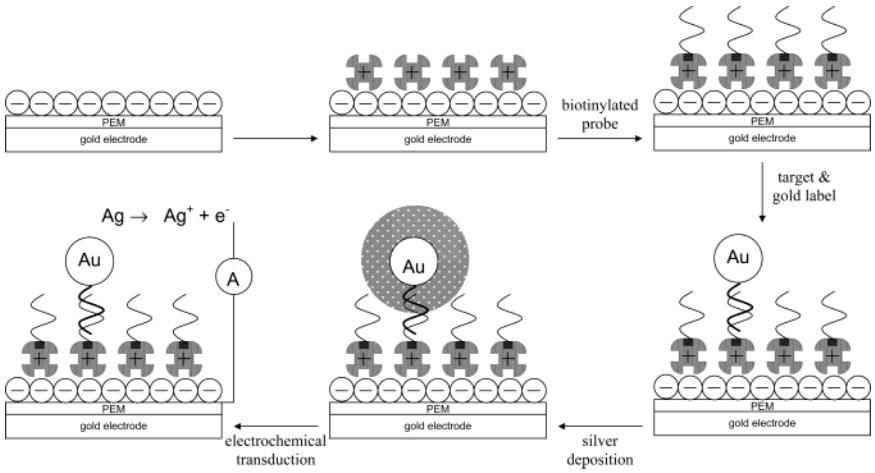

Figure 4. Schematic representation of the electrochemical DNA-hybridization detection using the silver-enhanced gold nanoparticle label on the gold electrode modified with polyelectrolytes, streptavidin, and the biotinylated probe.

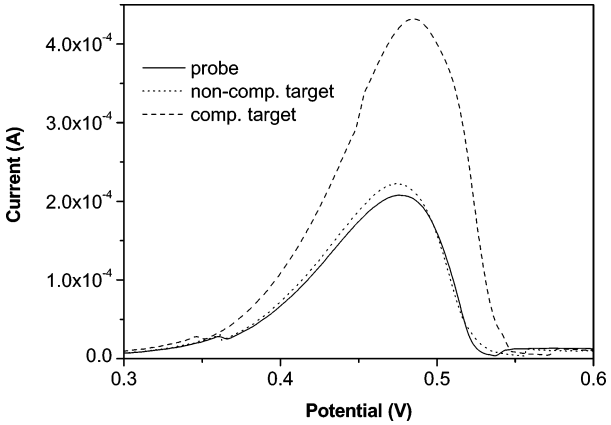

Figure 5. LSV scan of the gold electrodes (modified with the probe on the polyelectrolyte films) having different DNAs that were exposed to the silver-enhancement solution for 8 min.

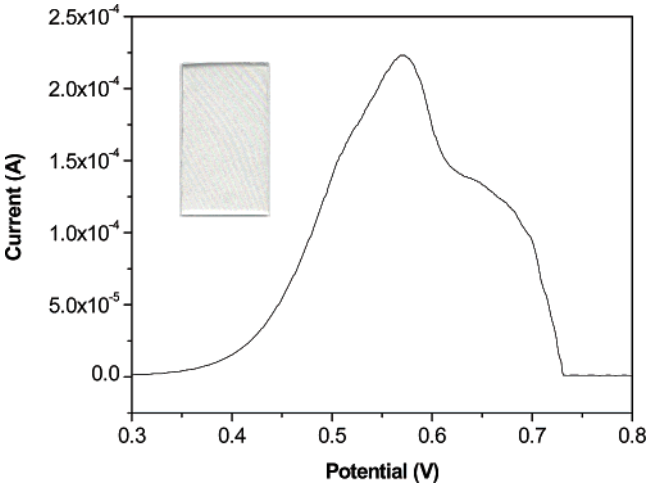

Figure 6. LSV scan of the ITO electrode (area of ~1.37 cm^2) subjected to silver enhancement for 5 min. Inset: image of the ITO electrode that remained transparent after exposure to the silver-enhancement solution.

(e.g., increments of 10 nM) with multiple samples at each concentration. Furthermore, the detection limit of the ITO electrodes with the silver-enhanced gold nanoparticle approach can be extended to the subnanomolar or even femtomolar range by lowering the background signal and incorporating other amplification routes with multiple nanoparticle tags (e.g., dendrimer and liposome).

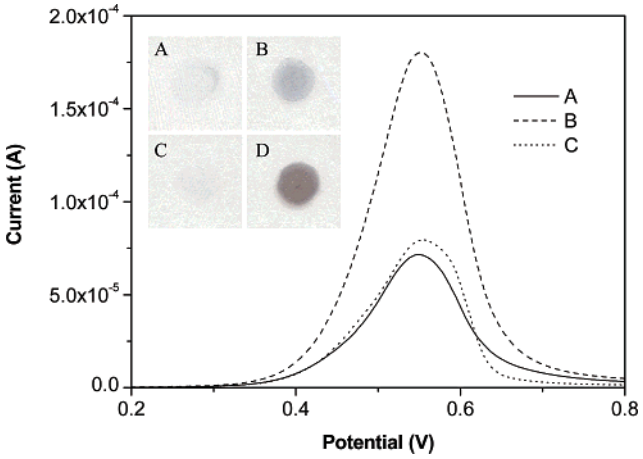

Figure 7. Insets: images of the ITO electrodes with different DNAs subjected to the silver enhancement with the (A) probe-only surface, (B) probe surface treated with the target, (C) probe surface treated with the negative control, and (D) positive-control surface. LSV scans of three of these surfaces (A−C) are given. The dimensions of the ITO electrodes used were 1 × 1 cm. The hybridizations with the target and negative control were carried out with a 1 μM solution in 2 × SSC buffer at room temperature for 1 h, followed by the incubation of the streptavidin/gold label for 1 h and silver enhancement for 8 min.

After the discussion of the implementation of the silver-enhanced gold nanoparticle approach on the gold and ITO electrodes, in the following text, general comments on the sensitivity and integration aspects are given. The signal-amplification capability of the silver-enhanced gold nanoparticle approach is manifested when compared with an electrochemical transduction of the hybridization event based on an electrochemically active intercalator. In a previous study,[9] gold electrodes modified with a probe (by the chemisorption of a 5′ thiol-modified probe) are used for sequence-specific detection of target DNA. Subsequent signal transduction is based on the preferential binding of the intercalator (Hoechst 33258) to the probe−target hybrid rather than to the single-stranded probe. The difference in charge between the probe-only surface and probe−target surface during the LSV scans for the gold electrodes is 0.03 mC (electrode-sensing area of 1.37 cm^2), whereas that for the ITO electrodes is 0.18 mC (sensing area of about 0.2 cm^2). After an area correction, the amount of silver is 40 times greater than that of Hoechst 33258.

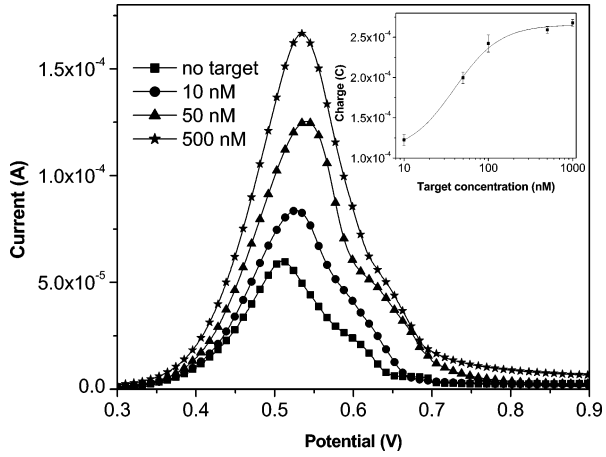

Figure 8. LSV scans of the ITO electrodes hybridized with different concentrations of the targets. Inset: calibration plot of the charge associated with the oxidation of the silver deposits on the gold nanoparticle label against the target DNA concentration. The charge was obtained by integrating the LSV curve with respect to time.

A striking feature of the detection platform described here is the heat stability of the immobilized probe on the electrode surface. The surface chemistry employed to immobilize the probes onto the gold (SAM of MUDA, polyelectrolyte multilayer films, avidin−biotin binding) and ITO (SAM of MPA, disulfide linkage) electrodes is stable below 100 °C. This thermal stability enables the direct integration of the detection platform with a polymerase chain reaction (PCR) microreactor. This integration would be an extension of our previous efforts on an integrated PCR microarray,[32] realizing the development of a portable microdevice for simultaneous DNA amplification and detection.

4. Conclusions

The electrochemical DNA-hybridization detection utilizing gold nanoparticle labels in combination with silver enhancement has been successfully demonstrated on the gold and ITO electrodes. For the gold electrode, a significant reduction in the background silver staining was achieved by modifying the electrode surface with polyelectrolyte multilayer films. The DNA probe was immobilized onto the $(PAH/PSS)_3$-modified gold electrode via an avidin−biotin interaction for the sequence-specific detection of target sequences. For the ITO electrode, its inherent low silver-deposition property was exploited to develop a sensitive DNA-detection platform. The electrode was modified with a SAM of MPA, to which a thiol-modified probe was attached through a disulfide linkage. These microfabrication-compatible electrode materials offer an ideal detection platform in miniaturized bioanalytical systems. In future work, we will implement the established silver-enhanced nanoparticle approach with the gold- or ITO-detection platform in an integrated PCR electrochemical cell microdevice for portable sequence-specific DNA analysis applications.

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