Immobilization of DNA onto Poly(dimethylsiloxane) Surfaces and Application to a Microelectrochemical Enzyme-Amplified DNA Hybridization Assay

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This paper describes immobilization of DNA onto the interior walls of poly(dimethylsiloxane) (PDMS) microsystems and its application to an enzyme-amplified electrochemical DNA assay. DNA immobilization was carried out by silanization of the PDMS surface with 3-mercaptopropyltrimethoxysilane to yield a thiol-terminated surface. 5′-acrylamide-modified DNA reacts with the pendant thiol groups to yield DNA-modified PDMS. Surface-immobilized DNA oligos serve as capture probes for target DNA. Biotin-labeled target DNA hybridizes to the PDMS-immobilized capture DNA, and subsequent introduction of alkaline phosphatase (AP) conjugated to streptavidin results in attachment of the enzyme to hybridized DNA. Electrochemical detection of DNA hybridization benefits from enzyme amplification. Specifically, AP converts electroinactive *p*-aminophenyl phosphate to electroactive *p*-aminophenol, which is detected using an indium tin oxide interdigitated array (IDA) electrode. The IDA electrode eliminates the need for a reference electrode and provides a steady-state current that is related to the concentration of hybridized DNA. At present, the limit of detection of the DNA target is 1 nM in a volume of 20 nL, which corresponds to 20 attomoles of DNA.

Introduction

We report a method for covalent immobilization of DNA directly onto a poly(dimethylsiloxane) (PDMS) microchannel surface and the application of this chemistry for carrying out a microelectrochemical enzyme-linked DNA hybridization assay. The facile surface immobilization of DNA gives rise to a strong and stable DNA linkage, and it demonstrates that the microfluidic channel itself can be used as an immobilization and reaction platform. This strategy provides an effective means for sensing DNA, because of the high surface-area-to-volume ratios of microchannels.

The use of microfluidic platforms for fabricating biosensors has attracted attention in recent years for the following reasons.[1−5] First, microfluidic devices hold out the promise of process integration, which includes sample pretreatment, preconcentration, separation, and detection.[1] Second, such devices are well-adapted for high-throughput analysis, portability, and handling small sample volumes.[1] Third, microdevice fabrication methods have evolved that rely on simple polymer processing.[4,6] In this latter regard, PDMS has received the most attention mainly because fabrication is exceedingly easy, it seals well to a number of other materials,[7] and because it is optically transparent.[8,9] However, PDMS is hydrophobic and thus prone to nonspecific adsorption, especially with respect to proteins, and this places some limits on its use for biosensing applications.[3,10]

There have been a number of reports involving the use of PDMS microchannel surfaces as platforms for immobilizing capture proteins that are relevant to the results described here. For example, Eteshola and Leckband adsorbed antibodies onto PDMS and showed that it was possible to carry out a sandwich enzyme-linked immunosorbent assay (ELISA) using fluorescence detection.[11] The de Rooij group described a three-layer coating composed of biotinylated IgG, neutravidin, and biotinylated antibodies that could be used for immunoassays.[12] Cremer et al. showed that capture antibodies could be immobilized on PDMS using a phospholipid bilayer,[13,14] and Langer's group used reactive polymer coatings to immobilize capture probes within PDMS channels.[15,16]

In addition to proteins, DNA capture probes have also been immobilized onto the walls of PDMS (and other polymeric) microchannels. For example, Kuhr et al. linked

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biotin to PDMS surfaces via a photopolymerization reaction, conjugated the surface-immobilized biotin to probe DNA, and then analyzed for target DNA using a fluorescence-based hybridization assay.[17] Wang et al. performed a similar sort of assay using capture DNA oligos immobilized on the surface of a poly(methyl methacrylate) (PMMA) microfluidic channel.[18] In contrast to these previous reports, our work focuses on direct attachment of DNA to PDMS microfluidic channels and the use of these PDMS-immobilized capture probes for implementing an electrochemical enzyme-amplified DNA assay.

Enzyme-amplified bioassays normally involve the use of capture DNA oligos immobilized on a solid support. The support provides a convenient means for separating reagents from the capture probes and for defining their location. Microbeads are commonly used for this purpose because they are commercially available, are easy to integrate into established detection methods, and have a high surface-area-to-volume ratio. However, packed beds of beads can lead to a high resistance for pressure-driven flow in microfluidic channels. As we show here, it is possible to immobilize close to the same number of probe oligos on the walls of a microfluidic reaction chamber as would be present on the surface of ~10-μm-diameter beads filling the same size reaction chamber.[19] Importantly, however, the pressure drop arising from the presence of beads within the channel is avoided when the capture probes are immobilized on the wall.

Here, an electrochemical enzyme-amplified hybridization assay[20,21] was used to detect the presence of a specific sequence of DNA. Although generally not as sensitive as fluorescence detection, electrochemical methods do have some important advantages. These include compatibility with miniaturization, simplicity, low cost, low power consumption, and easy integration with microfluidic systems.[22] Three strategies were used in this study to improve the electrochemical detection limit and to simplify the device configuration. First, signal amplification was accomplished using a well-established, robust alkaline phosphatase (AP) enzyme amplification approach, which involves conversion of electroinactive *p*-aminophenyl phosphate (PAPP) to electroactive *p*-aminophenol (PAP).[23−26] Because each enzyme converts many PAPP molecules to PAP, the signal resulting from hybridization is amplified. Second, the use of interdigitated array (IDA) electrodes eliminates the need for a reference electrode and provides a simple means for obtaining a steady-state current response, which is easier to analyze than a transient signal. Moreover, IDAs having a design rule smaller than that used in this study lead to redox recycling of the analyte and a corresponding amplification of the signal.[27] Third, probe DNA was directly immobilized on the surface of the PDMS microfluidic channel rather than on the IDA electrode. This has been previously shown to improve the performance of electrochemical enzyme-linked assays.[23] Specifically, when the capture chemistry is removed from the electrode it does not present a mass-transfer barrier to PAP nor is the immobilization chemistry subject to the instability that often arises from changes in electrode potential.

Experimental Section

Chemicals and Materials. 18-MΩ-cm water (Milli-Q, Millipore, Bedford, MA) was used to prepare aqueous solutions. PDMS precursors (Sylgard 184, Dow Corning, Midland, MI), indium-dope tin oxide (ITO) glass (Delta Technologies, Stillwater, MN), Alexa Fluor maleimide (A10254, Molecular Probes, Eugene, OR), DNA oligonucleotides (sequences provided later) modified with biotin, acrylamide, or fluorescein (Integrated DNA Technologies, Coralville, IA), 3-mercaptopropyltrimethoxysilane (MPS, Hüls Petrarch, Bristol, PA), tris(hydroxymethyl)aminomethane (Tris, Sigma, St. Louis, MO), and bovine serum albumin (BSA, Sigma) were used as received. Phosphate buffer saline (PBS) solutions consisted of 1.0 M NaCl, 20 mM Na_2HPO_4/NaH_2PO_4, 2.0 mM ethylenediaminetetraacetate (EDTA), and 0.2% sodium dodecyl sulfate (SDS). Tris buffer at pH 9.0 was prepared by titrating an aqueous solution containing 0.1 M Tris and 2.0 mM $MgSO_4$ with HCl or NaOH. PAPP was synthesized according to a literature procedure.[28] The DNA oligonucleotide sequences were as follows: 5′-acrylamide-modified capture DNA (5′-acrydite-PEG6-GTC AAT ACG GGA TAA TAC CG); 5′-biotinylated target DNA (5′-biotin-CGG TAT TAT CCC GTA TTG AC); and 5′-fluorescein-labeled DNA (5′-fluorescein-CGG TAT TAT CCC GTA TTG AC).

Fabrication of the DNA-Modified PDMS Monoliths. PDMS monoliths containing microfluidic channels were prepared following an established procedure.[7,29] Inlets and outlets were punched into the monolith using a needle. The monolith was then sonicated in ethanol for 1 min, sonicated in water for 1 min, and finally dried under a flowing stream of N_2. Next, it was placed in a plasma cleaner (PDC-32G, Harrick Scientific, Ossining, NY) and oxidized (in air) at medium power for 1 min. Immediately after removal from the plasma cleaner, the monolith was exposed to the HCl vapor above a concentrated, aqueous HCl solution for 10 s, and then it was exposed to MPS vapor (1.5 cm above the liquid MPS level) at 40 °C for 10 min. After curing at 80 °C in N_2 for 10 min, the MPS-modified monolith was allowed to react with 1.0×10^{-4} M acrylamide-modified capture DNA in a mixed DMF/H_2O (v/v, 80:20) solvent at room temperature for 2 h (top frame, Scheme 1).

Fabrication of the Interdigitated Array (IDA) Electrodes and the Microfluidic Devices. IDA electrodes were microfabricated on ITO-coated glass substrates using a previously described procedure.[29] The array consisted of two sets of six interdigitated ITO lines (25 μm wide) and spaces (13 μm) (Figure 1). The microfluidic device was fabricated by sealing the glass-supported IDA electrode to the DNA-modified PDMS monolith by applying pressure from a mechanical clamp. A typical fluidic system consisted of a 150-μm-wide channel and a 400-μm-wide incubation chamber, each 30 μm high. The channel and the incubation chamber surfaces were modified with DNA, but the surfaces of the inlet and the outlet were not.

Enzyme-Linked DNA Hybridization and Electrochemical Detection. The procedure used for enzyme-amplified electrochemical detection is illustrated in the second and third frames of Scheme 1, and the detailed experimental sequence is provided in Table 1. All operations were carried out at room temperature (22 ± 1 °C). The IDA current was measured using

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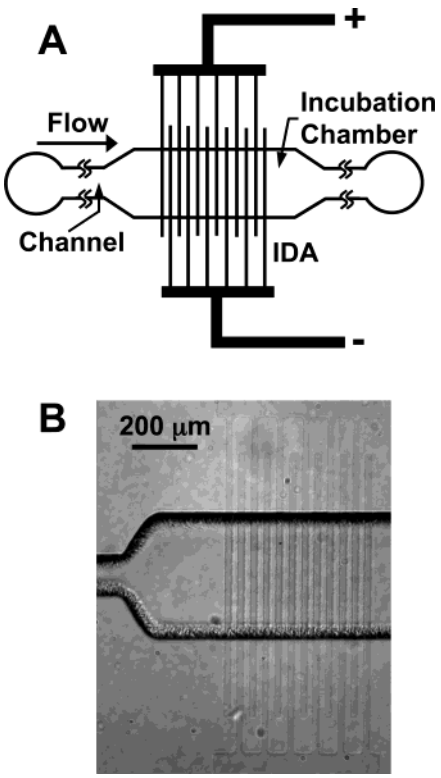
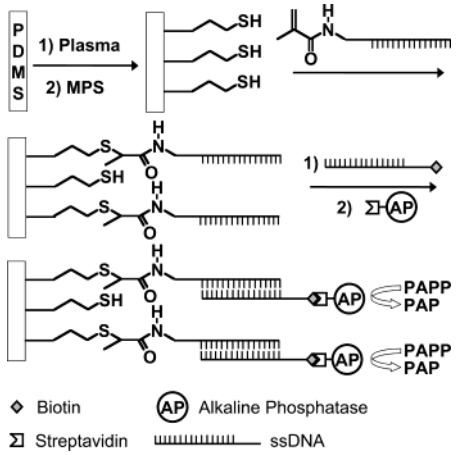


Figure 1. (A) Schematic representation of the PDMS microelectrochemical device used in this work. The dimensions of the incubation chamber are length, 1500 μm; width, 400 μm; and height, 30 μm. (B) An optical micrograph of the incubation chamber containing the IDA electrode.

Scheme 1



a potentiostat (model CHI660A, CH Instruments, Austin, TX). Cyclic voltammetric experiments were performed in a conventional three-electrode cell (Pt counter electrode and Ag/AgCl, 3 M NaCl reference electrode) outfitted with a single ITO working electrode having an area of 0.015 cm^2.

Fluorescent Labeling and Imaging. Fluorescence images were acquired using a microscope (Eclipse TE 300, Nikon, Japan) equipped with a filter block (480 nm for excitation and a 510-nm long-pass for emission), a 100-W Hg lamp, and a charge-coupled device (CCD) camera (SenSys 1401E, Photometrics, Tucson, AZ). A MPS-modified PDMS surface was imaged after it was reacted with 0.1 mM Alexa Fluor maleimide in DMF for 60 min, rinsed with DMF, and blown dry with N_2. Similarly, a DNA-modified PDMS surface was imaged after it was processed in the manner described earlier for the DNA assay, except 8 μM fluorescein-modified DNA was hybridized to the surface-confined probe in place of biotin-modified target DNA. The surface density of hybridized, fluorescent DNA was estimated by comparison of its fluorescence intensity to that of a standard consisting of a 22-μm-high microchannel filled with a 1.0 μM solution of the same fluorescent DNA.

Table 1. Experimental Procedure Used for the Enzyme-Amplified, Microelectrochemical DNA Assay[a]

1. Flow PBS buffer for 10 min.
2. Inject target DNA in PBS buffer for 10 min.
3. Incubate for 30 min.
4. Flow PBS buffer for 10 min.
5. Inject BSA solution (5.0 mg/mL) in Tris buffer for 10 min.
6. Incubate for 30 min.
7. Flow Tris buffer for 10 min.
8. Inject 0.1 mg/mL of streptavidin−alkaline phosphatase conjugate in Tris buffer for 10 min.
9. Incubate for 30 min.
10. Flow Tris buffer for 10 min.
11. Inject 5 mM PAPP in Tris buffer for 10 min.
12. Incubate for variable times (0−60 min).
13. Detect electrochemically for 1 min.

[a] The flow rate for all reagents and wash solutions was 2.0 μL/min. Phosphate buffer saline (PBS) solutions consisted of 1.0 M NaCl, 20 mM Na_2HPO_4/NaH_2PO_4, 2.0 mM EDTA, and 0.2% sodium dodecyl sulfate (SDS). Tris buffer at pH 9.0 was prepared by titrating an aqueous solution containing 0.1 M Tris and 2.0 mM $MgSO_4$ with HCl or NaOH.

Results and Discussion

Immobilization of DNA on the Surface of PDMS Monoliths. As shown in Scheme 1, the immobilization of DNA onto a PDMS surface involves three steps: plasma-induced oxidation of the PDMS surface, functionalization of the oxidized surface with a silane coupling agent bearing a distal thiol group, and subsequent reaction of the thiol groups with acrylamide-modified DNA. The silanization step was carried out using a vapor-phase reaction method.[30] As described in detail in the Experimental Section, plasma-treated PDMS was exposed first to HCl vapor and then to MPS vapor. The acid acts as a catalyst that increases the rate of MPS immobilization on the PDMS surface. The optimum MPS reaction time was found to be ~10 min; longer times led to decreased efficiency of the subsequent reaction with DNA.

Following vapor-phase silanization, the presence of thiol groups on the surface of the PDMS monolith was indirectly verified by immobilization of the thiol-reactive fluorescent dye Alexa Fluor maleimide. Figure 2A shows a fluorescence micrograph of an MPS-modified PDMS surface after bringing the left side of the substrate into contact with a solution containing the dye and then rinsing with solvent. The appearance of fluorescence on this part of the surface indicates thiol modification of PDMS induces dye immobilization. When the same set of surface reactions was carried out, except for omitting MPS immobilization, the PDMS surface showed no sign of Alexa Fluor maleimide fluorescence (data not shown).

Figure 2B shows a fluorescence micrograph of MPS-modified PDMS after exposing the right side of the surface to acrylamide-modified probe DNA and then exposing the entire surface to complementary, fluorescein-labeled target DNA. Fluorescence was detected only from the probe-DNA-modified region of the substrate. This result indicates that the capture oligo is present on the surface, that it is able to hybridize with the target oligo, and that there is little nonspecific adsorption of the target on the probe-free fraction of the surface. A control experiment was carried out in which the plasma-treated (but MPS-free) surface was sequentially exposed first to acrylamide-modified DNA and then to fluorescein-labeled, complementary target DNA. In this case, no detectable fluores-

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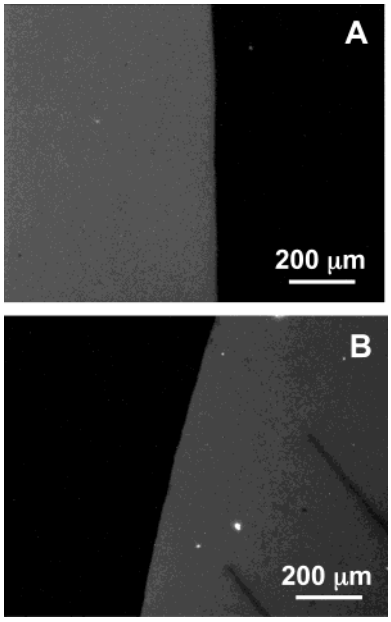

Figure 2. Fluorescence micrographs of MPS-modified PDMS surfaces. (A) After exposure of the left side of an MPS-modified surface to the thiol-reactive fluorescent dye Alexa Fluor maleimide (the full gray scale is 320 counts/s). (B) After exposure of the right side of an MPS-modified PDMS surface to acrylamide-modified probe DNA and then exposure of the entire surface to complementary, fluorescein-labeled target DNA. The full gray scale is 120 counts/s.

cence was observed. Taken together, these results provide evidence that capture DNA modified on its 5′ end with acrylamide can be covalently attached to the thiolated PDMS surface via a Michael addition reaction. Although it is not possible to unambiguously confirm covalent attachment, this conclusion is bolstered by previously reported findings that a thiol-acrylamide Michael addition reaction leads to immobilization of DNA onto other surfaces such as glass.[31,32]

A variety of techniques have been used to quantify the surface density of immobilized DNA, including X-ray photoelectron spectroscopy (XPS) and Fourier transform infrared (FTIR) spectroscopy.[33,34] However, these methods cannot be used to measure the surface density of immobilized DNA on PDMS because of spectral interferences. Therefore, the DNA surface density was determined by hybridizing fluorescein-labeled DNA to the PDMS-immobilized probe DNA, and the intensity was compared to a standard (details are provided in the Experimental Section). The result is that measurements made using 10 independently prepared PDMS substrates yielded a surface density of hybridized DNA of $(4.0 \pm 1.9) \times 10^{11}$ targets/cm^2. This value is lower than those reported using similar immobilization strategies. For example, Tarlov used a Au substrate and found a surface density on the order of 10^{13} DNA/cm^2.[33] Corn has measured the surface density of DNA immobilized on Au and found it to be 1.5×10^{12} DNA/cm^2.[35,36] As mentioned in the Introduction, others have immobilized DNA onto PDMS and related materials using indirect attachment protocols, but they have not reported quantitative hybridization efficiencies.[17,18]

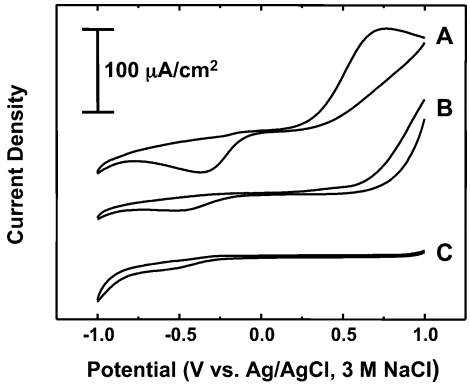

Figure 3. Cyclic voltammograms obtained using a conventional, three-electrode electrochemical cell consisting of an ITO working electrode (area = 0.015 cm^2), a Ag/AgCl, 3 M NaCl reference electrode, and a Pt counter electrode. The electrolyte solution contained 0.1 M, pH 9.0 Tris buffer, 2.0 mM $MgSO_4$, and (A) 1.0 mM PAP, (B) 5.0 mM PAPP, and (C) no redox-active couple. The scan rate was 100 mV/s.

Microelectrochemical Enzyme-Amplified DNA Hybridization Assays. Enzyme-amplified assays are well-known methods for the analysis of proteins, DNA, and other biomolecules,[37,38] and this method has frequently been coupled with electrochemical detection of the product of the enzymatic reaction.[20,21,23,26,27] The objective of this part of our study is to show that this well-established DNA detection method can be integrated into a PDMS-based microfluidic format. Specifically, DNA capture oligos were directly immobilized onto the PDMS surface, as described in the previous section, and then hybridization was signaled by measurement of the faradaic current resulting from the redox chemistry of PAP at an IDA electrode.

Prior to using PAP as a reporter of DNA hybridization in microfluidic devices, we first examined its electrochemical behavior in a conventional, three-electrode cell outfitted with a single, macroscopic ITO electrode. Figure 3A shows a cyclic voltammogram (CV) obtained in a solution containing 0.1 M, pH 9.0 Tris buffer, 2.0 mM $MgSO_4$, and 1.0 mM PAP. Compared to the nearly thermodynamically reversible behavior previously reported for PAP at glassy carbon electrodes,[39] PAP voltammetry at ITO electrodes is far more sluggish (the difference between the anodic and cathodic peak potentials, ΔE_p, is 1.13 V). Nevertheless, the presence of both oxidation and reduction peaks indicates a quasireversible electron-transfer process,[40] which suggests that a sufficient potential difference applied to the IDA electrode will result in redox recycling.[41] We note that from a redox-recycling perspective it would be preferable to use a glassy carbon IDA electrode to access the faster electron-transfer kinetics of PAP.[42] However, carbon electrodes are not easily adapted to polymeric microfluidic systems.[43]

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Figure 3B compares the voltammetry of 5.0 mM PAPP to 1.0 mM PAP (Figure 3A) under identical electrochemical conditions. This comparison is important because, following a typical assay, the concentration of PAPP remains higher than the concentration of PAP. Accordingly, it is essential that the potential difference between the IDA electrodes be sufficient to oxidize PAP and reduce *p*-quinoneimine (PQI, the oxidation product of PAP) but not so extreme as to oxidize or reduce PAPP. The CVs in Figure 3A and B indicate that the ratio of the PAP-to-PAPP anodic current (after correcting the CVs to account for the higher PAPP concentration) is optimal and equal to ∼46 at a potential of 0.54 V. Therefore, even though the electron-transfer kinetics of PAP are sluggish on ITO, it should be possible to amplify the signal resulting from DNA hybridization using a linked AP enzyme. For completeness, Figure 3C shows a CV obtained under conditions identical to those used in parts A and B of Figure 3 except in the absence of PAP and PAPP. In the important potential region between 0 and +1.0 V, the background current is very low. The relatively small cathodic current between 0 and −1.0 V probably arises from kinetically slow proton reduction on ITO.

The just-discussed voltammetry data provides a basis for selecting the optimal IDA-electrode bias voltage for the reference-electrode-free microelectrochemical detection of PAP in the presence of PAPP. Accordingly, we carried out two-electrode electrochemical experiments in the PDMS microfluidic system using IDA electrodes and the same solutions used to obtain the data in parts A and B of Figure 3. These concentrations of PAP and PAPP were chosen to simulate conditions that might arise following DNA hybridization and enzymatic conversion of PAPP to PAP in a real assay. The data, which are provided in the Supporting Information (Figure S1), reveal that the highest current ratio (1.0 mM PAP vs 5.0 mM PAPP) is observed at a bias voltage of ∼0.7 V. Accordingly, this bias was used for detecting DNA hybridization in the experiments described next.

Phosphate buffer saline (PBS, pH 7.4) solution was used as the buffer for DNA hybridization.[44] Tris buffer containing 2.0 mM Mg^{2+} was selected for the enzyme amplification reaction and the electrochemical detection steps, however, because it provides a larger pseudo-rate-constant (V_{max}/K_m) for AP compared to those of other buffers.[45] It was necessary to optimize the pH of the Tris buffer because the enzyme turnover number (TON) and nonenzymatic hydrolysis of PAPP, which occur simultaneously in the assay described here, are best carried out in different pH ranges. Specifically, a moderately high pH value (pH ∼ 10) results in the highest TON for AP,[46] but nonenzymatic hydrolysis of PAPP to PAP, which is significant at an alkaline pH, leads to an increased background signal.[46] Additionally, oxidation of PAP by oxygen in air proceeds more quickly at a high pH, and this imposes a limitation on the total incubation time of the enzymatic reaction and thus increases the limit of detection.[24,45]

This pH study was carried out in the microelectrochemical device configured with an IDA electrode using two kinds of solutions: one was composed of 2 μg/mL of AP and 5.0 mM PAPP, and the other was a control solution containing only 5.0 mM PAPP in Tris buffer or a three-component buffer containing 3-(cyclohexylamino)-1-propanesulfonic acid (CAPS), 2-(cyclohexylamino)ethanesulfonic acid (CHES), and *N*-[tris(hydroxymethyl)methyl]-3-aminopropanesulfonic acid (TAPS). After 30 min, the amount of PAP in the reaction chamber was determined by applying the optimized bias voltage (0.7 V) and monitoring the current arising from the IDA electrode for 1 min. The highest ratio of the IDA-electrode current for the enzyme-plus-PAPP solution, compared to the PAPP-only solution, determined over a pH range of 8.0−10.0, was found to arise for the pH 9.0 Tris buffer (Supporting Information, Figure S2).

After optimizing the bias voltage and buffer, the enzyme-amplified DNA hybridization assay was carried out in the microfluidic cell. In these experiments, the sequence of steps shown in Table 1 was followed and only the concentration of target DNA was varied. The solution introduction and washing steps were carried out using a pressure-driven pump operating at a flow rate of 2.0 μL min^{-1}. Before describing the results, it is important to note that nonspecific adsorption (NSA) of the enzyme label is often a problem for enzyme-amplified assays of the type used here. Indeed, control experiments indicated that some AP does adsorb to the interior walls of the PDMS microdevice. However, the extent of NSA was reduced significantly by coating the interior surface with bovine serum albumin (BSA) prior to enzyme conjugation. Specifically, BSA passivation resulted in a decrease of the background IDA current from 12 to 3 nA (after subtraction of the ∼1-nA IDA current that flows in the absence of AP) when the DNA-modified microreactor was exposed to AP for 30 min, rinsed with buffer for 10 min, and then used for PAPP conversion to PAP (this experiment was carried out on a single-stranded-DNA-modified substrate prior to hybridization; see the Supporting Information, Figure S3).

Figure 4A shows the IDA-electrode current as a function of the time allotted for incubation of PAPP with AP for a target DNA concentration of 100 nM. Figure 4B shows a plot of the steady-state IDA current in Figure 4A as a function of incubation time. It shows that the IDA signal increase is approximately linear for the first 30 min after PAPP injection but that at longer times the IDA current slightly decreases. The slower rate of current increase toward the end of the 1-h incubation period might be related to the instability of PAP. Specifically, it has been reported that PAP is oxidized in air on a time scale of minutes in aqueous solution. This problem is especially acute at basic pHs.[47] The optimal incubation time of 30 min used here is comparable to that reported by Heineman and co-workers, who suggested that the incubation time of PAPP should be restricted to <15 min to minimize air oxidation.[24]

Figure 4C shows the IDA current as a function of the target DNA concentration after an incubation time of 30 min. Under the present experimental conditions, the limit of detection for target DNA is ∼1 nM and the limit of quantitation (LOQ), which is 3 times the background signal, is 10 nM. The limit of detection depends on several factors. First, NSA of enzyme onto the PDMS microchannel surface will result in formation of PAP. This effect can be minimized by coating the walls with BSA or other inhibitors, but it cannot be eliminated. Second, low concentrations of target DNA will naturally yield relatively low concentrations of PAP over the time period allotted for incubation. Because of the finite overlap of the PAP

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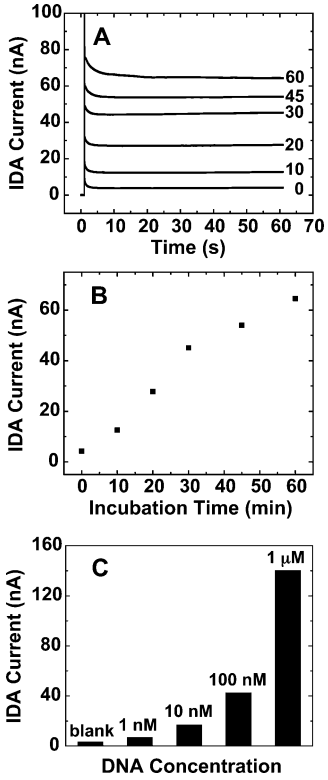

Figure 4. Electrochemical detection of DNA via redox recycling of PAP at an IDA electrode in a PDMS microelectrochemical system. (A) IDA-electrode current as a function of time for a 5.0 mM PAPP solution incubated with AP for the times (in minutes) listed to the right of the current transients. The target DNA concentration was 100 nM. At $t = 0$, the bias voltage was stepped from 0 to 0.7 V. (B) A plot of the steady-state IDA currents from part A as a function of incubation time. (C) A plot of the steady-state IDA-electrode current as a function of the concentration of target DNA used for hybridization. The incubation time was 30 min, and the data were background-corrected by subtracting the IDA current at the start of incubation. In all cases, the electrolyte solution contained 0.1 M, pH 9.0 Tris buffer and 2.0 mM $MgSO_4$.

and PAPP reduction potentials (parts A and B of Figure 3), this situation will lead to a significant background current arising from the presence of PAPP. A substrate/product pair with a larger difference between their reduction potentials would solve this problem. Third, the efficiency of redox recycling is determined by the width of the IDA fingers and the spacing between them. Here, these values are both relatively large (lines, 25 μm; spacing, 13 μm), and the degree of amplification arising from this configuration is very small (Supporting Information). The main advantage of the IDA in our experiments is that it provides a means for obtaining a steady-state current response and it eliminates the need for a reference electrode. However, an order of magnitude decrease in the design rule for the IDAs would be required to realize significant feedback amplification from redox recycling.[27]

Figure 4C also indicates that the IDA signal after 30 min of incubation time does not increase linearly with respect to the DNA concentration. This behavior is anticipated if the binding isotherm of DNA is nonlinear, which is common for surface-based assays.[48] In other words, DNA capture approaches saturation at higher concentrations of target DNA due to the fact that there are a finite number of DNA capture oligos immobilized on the PDMS surface. Such nonlinear signals for electrochemical ELISA assays carried out within microfluidic systems have also been observed by Choi et al.[26]

Summary and Conclusions

Here, we have shown that DNA can be directly immobilized on the surface of PDMS microdevices and that this provides a means for carrying out enzyme-amplified DNA hybridization assays. There are two significant outcomes of this work. First, we have shown that a PDMS surface can be thiolated using a simple vapor-phase reaction. In addition to the DNA assay reported here, we anticipate that thiol modification of PDMS will be useful for immobilizing other reagents and capture probes as well as for inducing adhesion of metals and semiconductors to PDMS. Moreover, the PDMS-modification chemistry is simple and should be compatible with nucleophilic pendent functional groups such as NH_2 and SH. Second, an enzyme-amplified DNA hybridization assay has been integrated into a PDMS microfluidic device using PDMS-immobilized DNA as the capture probe and an IDA electrode as the detector. This reference-electrode-free electrochemical configuration is advantageous for analysis within microchannels where the implementation of a three-electrode system is difficult due to the limited space and the complexity of fabrication. At present, the limit of detection of the device described here is 1 nM DNA, which corresponds to 20 attomoles within the 20-nL incubation chamber. It might be possible to reduce these values after further optimization of the experimental conditions.

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Supporting Information Available: Figures showing optimization of the bias voltage applied to the IDA electrodes, optimization of the buffer composition and pH for the AP-catalyzed conversion of PAPP to PAP and for the electrochemical detection of PAP, the effect of BSA passivation on the nonspecific adsorption of AP onto a DNA-modified PDMS microfluidic surface, and a discussion of the extent of redox recycling using the IDA electrodes described in this paper. This information is available free of charge via the Internet at http://pubs.acs.org.

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