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Publisher's version / Version de l'éditeur:
https://doi.org/10.1021/ac060486n
Analytical Chemistry, 78, 13, pp. 4457-4464, 2006-05-18

PCR-Free DNA Detection Using a Magnetic Bead-Supported Polymeric Transducer and Microelectromagnetic Traps

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A fluorescent polymeric hybridization transducer supported on magnetic microbeads was investigated for the rapid, ultrasensitive, and sequence-specific detection of DNA. We show that the polymer derivative can be used to detect target DNA directly on magnetic particles by preparing "target-ready" microbeads grafted with the polymer and suitable DNA probes. A detection limit of ~200 target copies in a probed volume of 150 μL (1.4 copies/μL) was obtained for a DNA sequence specific to *Candida albicans* This detection scheme does not require the release of the hybridized target DNA prior to its detection or the labeling or amplification of the nucleic acids. Furthermore, we show that the fluorescence from these biosensing magnetic beads can be read while magnetically confined in a small volume by a microelectromagnetic trap, which offers the possibility of performing both the preconcentration and detection steps simultaneously on the same support. The combination of the fluorescent polymer biosensor with magnetic particle-assisted DNA preconcentration extends the application of this ultrasensitive biosensor to biological samples with complex matrixes and to integrated lab-on-a-chip platforms, where it could be used for fast multitarget DNA detection in point-of-care diagnostics and field analysis.

Interest in the development of fast and reliable sequence-specific DNA biosensors has grown tremendously over the past few years, fueled by the significant advantages that they could provide in various fields such as the diagnosis of infections,[1,2] the identification of genetic modifications,[3] forensic analysis,[4] and proteomic investigations.[5,6] Because the amount of DNA material to be detected is often extremely small, the need for a detection scheme capable of transducing the hybridization event with sufficient sensitivity has led to the investigation of a number of sensitive approaches based mostly on optical,[5,7-20] electrochemical,[21-25] or magnetoresistive[26,27] detection. Relatively few of these methods, however, offer the simultaneous advantages of rapidity, simplicity, specificity, and detection sensitivity without the use of chemical tagging of the DNA target or polymerase chain reaction[28] (PCR) amplification, which increase the assay's turnaround time and may limit quantitative analysis. Moreover, in the case of blood infections, the amount of human (genomic) DNA can be 10^{14} times

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10.1021/ac060486n CCC: $33.50
Published on Web 05/18/2006

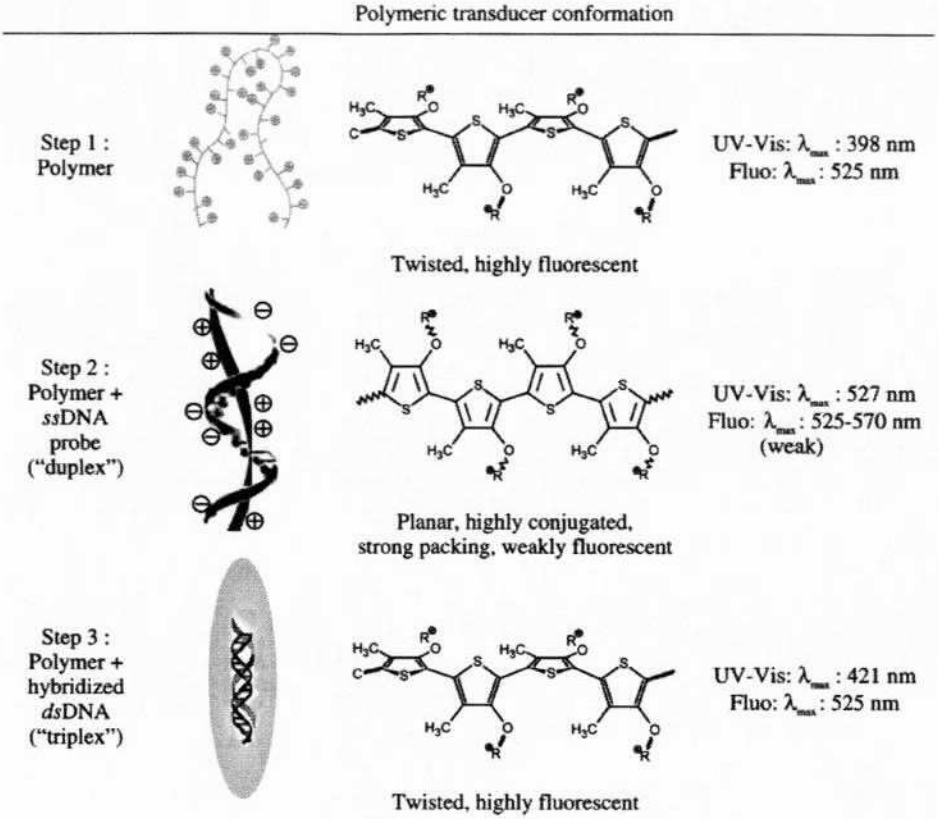

Figure 1. Principle of DNA detection by polymeric transducer. A cationic water-soluble polymer (step 1) is electrostatically bound to a negatively charged DNA probe to form weakly fluorescent duplex species (step 2) which, in the presence of perfectly complementary DNA target, forms highly fluorescent triplex species (step 3).

more abundant than the viral or bacterial target DNA, which represents an important challenge in terms of selectivity.[1]

One strategy that has increased in popularity in recent years is the use of surface-functionalized magnetic micro- or nanoparticles to selectively bind low-abundance target analytes (DNA, bacteria, or virus) and preconcentrate them and to discard the sample matrix prior to the detection step.[22,29-32] These particles, available across a wide size range, offer large contact surfaces and functionalized surface densities, thus allowing the optimization of operational and separation procedures with relative ease. For instance, Nam et al.[5] reported a procedure based on the sandwiching of target DNA with probe-functionalized magnetic microparticles and DNA-modified gold nanoparticles to separate the target DNA from the sample matrix and amplify the signal by releasing so-called *bar-code DNA* which is then detected using a chip-based silver metallization technique. Xu et al.[33] recently reported the selective identification of target DNA using a magnetically assisted DNA detection platform based on magnetic particle preconcentration. In both cases, detection of the target DNA first requires the release of captured target DNA by dehybridization, which tends to lengthen the analysis time and dilute the sample. Both issues could be resolved by combining target capture on magnetic particles and detection of the hybridization event. Previous reports have shown the possibility of effectively manipulating and controlling the motion of magnetic microbeads using microfabricated electromagnets (ref 34 and references within). Microelectromagnets not only produce strong local magnetic fields but can also be easily switched on and off by controlling the electrical current. The possibility of magnetically confining and concentrating target analytes in a microscopic volume for in situ optical detection offers great potential for the detection of minute amounts of target DNA and, to the best of our knowledge, has not yet been demonstrated experimentally.

Notable advances were recently reported on the use of conjugated polymers as hybridization transducers for the rapid and simple detection of DNA.[15] In particular, a cationic polythiophene derivative was shown to provide zeptomole (10^{-21} mole) detection levels and the capability to distinguish perfect matches from oligonucleotides having as little as a single base mismatch or SNPs (single nucleotide polymorphism).[14] The detection strategy relies on the different conformations adopted by the polymer transducer when electrostatically bound to either ssDNA (single-stranded DNA) or dsDNA (double-stranded DNA) and on the difference in optical properties (absorption and fluorescence) between the two conformations (see Figure 1). This behavior, known as chromism, is well documented for this class of cationic

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Table 1. Sequences of Oligonucleotides Used in This Work[a]

base sequence		name
C. albicans (20-mers)		
5′-biotin-C6 spacer-CATGATTGAACCATCCACCA-3′	probe	X1
3′-GTACTAACTTGGTAGGTGGT-5′	target	Y1
3′-GTACTAACTTGGTAGGTGGT-Cy3-5′	target tagged	Y1-Cy3
3′-GTACTAACTTCGTAGGTGGT-5′	one mismatch	W1
3′-GTACTAACTTCGAAGGTGGT-5′	two mismatches	W2
B. anthracis (25-mers)		
5′-biotin-C6 spacer-TTGATTATTGTTATCCTGTTATGCC-3′	probe	X2
3′-AACTAATAACAATAGGACAATACGG-5′	target	Y2

[a] Bold characters denote a mismatch with the probe sequence X1 (see text).

polymers[10,35] and is mainly caused by a change in the delocalization of π electrons along the carbon chain backbone that occurs when switching between the two conformations. Thus, by monitoring, via the change in fluorescence intensity, the hybridization of the complementary ssDNA target with the so-called "duplex" (polymer + *ss*DNA capture probe), as little as 220 complementary oligonucleotide target molecules could be detected in a 150 μL sample volume (0.36 zmol) in less than 1 h.[14]

This ultrasensitive detection scheme, which had until now only been demonstrated for purified DNA samples dispersed in homogeneous aqueous media, can be coupled to magnetic particle-assisted DNA preconcentration to extend its application to real biological samples having complex matrixes. Thus, the purpose of the present work is to develop a rapid and sensitive technique for the sequence-specific detection of DNA based on the electromagnetic confinement of particle-bound target DNA and optical detection of the target using a fluorescent polythiophene derivative. This new approach is first demonstrated in a "capture and release" scheme, in which the target DNA is captured by functionalized magnetic microbeads, separated from the sample matrix, and then released in a suitable aqueous media prior to detection with the polymer-probe duplex. Furthermore, it is shown herein how the polymer derivative can be used to detect target DNA directly on magnetic particles by preparing polymer + probe "target-ready" microbeads. This new detection scheme eliminates the need to first release the hybridized target DNA prior to optical detection, as well as the need for any chemical manipulation of the nucleic acids (i.e., labeling). To the best of our knowledge, this is also the first demonstration of the use of a polymer hybridization transducer for optical detection of DNA on solid substrates. Finally, it is shown that the fluorescence from the polymer-complexed DNA can be read while the microbeads are being magnetically confined in a small volume by a microelectromagnetic trap (μ-EMT). This offers the possibility to increase the signal-to-background ratio and decrease the signal noise from concomitant species, as well as the possibility of having the preconcentration/purification and detection steps occur on the same support.[36]

EXPERIMENTAL SECTION

Materials. Paramagnetic streptavidin-functionalized microbeads (Dynabeads M-280, 2.8 μm diameter) were purchased from Dynal Biotech (Oslo, Norway) in a 10 mg beads/mL suspension. Hydrogenated Triton X-100 (reduced), Tween 20, sodium citrate, lithium chloride, sodium chloride, sodium hydroxide, ethylenediaminetetraacetic acid disodium salt (Na_2EDTA), and tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl) were all purchased in the highest quality grade available from Aldrich (Oakville, ON, Canada). All dilutions and solution handling were performed in sterile plasticware, and all solutions were prepared using sterile Nanopure water. Oligonucleotides were all purchased from Integrated DNA technologies, Inc. (Coralville, IA) in a standard desalting purification grade, except for the labeled target Y1-Cy3 (see Table 1 for DNA sequence details) which was purchased from TriLink Biotechnologies Inc. (San Diego, CA) in a double RP-HPLC purification grade. Two biotin-modified capture probes were covalently anchored on the microbeads. The first one (X1) is a 20-mer sequence corresponding to a conserved region of the *Candida albicans* yeast genome. Y1 is the corresponding target to X1 (perfect match), while W1 contains one mismatch and W2 contains two mismatches with respect to the X1 capture probe (W2 also corresponds to a *Candida dubliensis*-specific sequence). The second biotinylated capture probe (X2) is a 25-mer sequence specific to *Bacillus anthracis*, with Y2 its corresponding target (perfect match). We also used non-biotinylated X1 and X2 oligonucleotide probes for complexation with the cationic polythiophene, the synthesis procedure of which has been described elsewhere.[10] The human genomic DNA used for these experiments was extracted from patient blood (as described elsewhere[37]), stored at −20 °C until use, and aliquoted to the desired concentration (33 000 copies/μL determined by UV absorption at 260 nm in TE 1× buffer (10 mM Tris, 1 mM EDTA)). Different buffer solutions were used throughout the present work: TTL (100 mM Tris-HCl (pH 8.0), 0.1% Tween 20, 1 M LiCl), TT (250 mM Tris-HCl (pH 8.0), 0.1% Tween 20), TTE (250 mM Tris-HCl (pH 8.0), 0.1% Tween 20, 20 mM Na_2EDTA), and hybridization mixture (750 mM sodium citrate, 150 mM NaCl).

Bead Functionalization and Characterization. Prior to the conjugation of the biotinylated DNA probes with the streptavidin-functionalized microbeads, the latter were first magnetically separated, decanted, washed twice with TTL buffer, and resuspended in TTL, following the standard procedure described by Fry et al.[30] The conjugation step was performed by mixing 1 nmol of probes with 1 mg of microbeads in a final volume of 24 μL (in TTL buffer); the mixture was left under gentle shaking for 20 min

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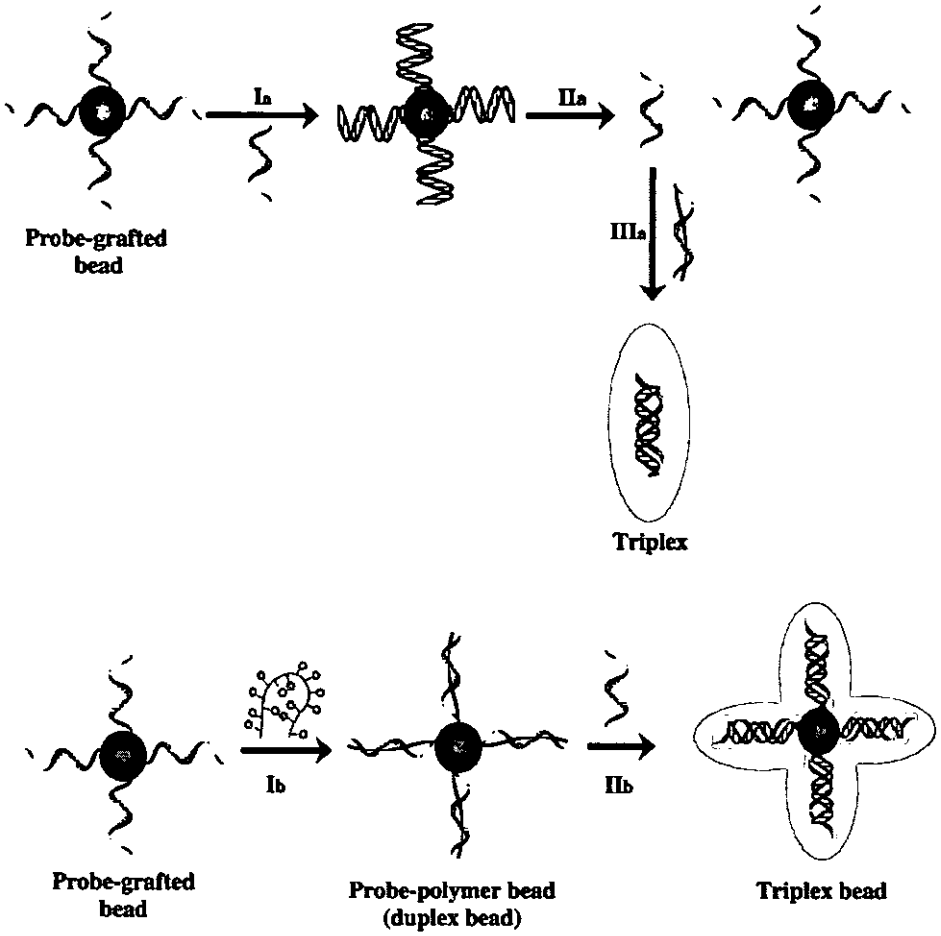

Figure 2. Polymer–magnetic bead detection schemes. Capture and release (top): (Ia) addition of target DNA, (IIa) thermal release of target DNA (80 °C), and (IIIa) addition of duplex and detection of fluorescence. On-bead detection (bottom): (Ib) addition of polymer and (IIb) addition of target DNA and fluorescence detection.

at room temperature. The functionalized beads were then pulled to the side of the reaction vial with a rare earth magnet and washed in 0.15 M NaOH to remove any residual oligonucleotide probes. This washing procedure was repeated twice with TT buffer. The beads were incubated at 80 °C for 10 min in TTE buffer to remove any unstable biotin-streptavidin couplings; then they were washed twice with 50 μL of TTE and finally resuspended in 100 μL of TT buffer. With this sample preparation procedure, the probe–microbead conjugates were shown to remain stable for a period of up to 2 months, under proper storage and handling conditions.

The binding capacity of the microbeads was determined experimentally by measuring the amount of biotinylated probes left in the supernatant after the conjugation step (UV absorption at 260 nm, performed on a Cary 500 Scan, Varian Inc., Palo Alto, CA) and was found to be 500 pmol of 20-mer biotinylated probes/mg of microbeads, which is within the range stated by the manufacturer (200–700 pmol/mg). The hybridization capacity was determined by fluorescence measurements of the supernatant and subsequent washing solutions on a commercial spectrofluorimeter (Cary Eclipse, Varian Inc.), using a labeled target DNA (Y1-Cy3). The results show that a maximum of 250 pmol of complementary target can be captured per milligram of probe-functionalized microbeads (up to 2×10^6 hybridization sites per bead) and that the captured target DNA is quantitatively released upon thermal denaturation, in accordance with previous reports.[30]

DNA Detection by Capture and Release. Probe-grafted microbeads (62.5 μg, 4×10^6 beads) were resuspended in 1 mL of hybridization buffer with 6×10^4 target ssDNA strands (1 μL, 10^{-13} M), and this mixture was allowed to react for 1 h at 50 °C under gentle shaking. The beads were decanted, separated, washed twice with 50 μL TT, and resuspended in 10 μL of pure water for the release step (4 min, 80 °C). After the beads were decanted and washed with 10 μL of hot (80 °C) pure water, the supernatant was collected. The solution obtained with this procedure contained purified and preconcentrated target ssDNA (100-fold preconcentration), which was measured quantitatively using the polymeric transducer (Figure 2a) and a custom-made fluorimeter, as described in detail elsewhere.[14] For all quantitative measurements performed with this fluorimeter (i.e., capture and release and on-bead detection), each fluorescence data point in the calibration curves is the average of 10 optical measurements on a given sample, and each measurement was obtained by the integration of the fluorescence signal over a period of 10 s. The detection limit was calculated as 3 times the standard deviation of the optical measurements for the blank signal divided by the slope of the calibration curve.

On-Bead DNA Detection in Suspension (3 mL cuvettes). ssDNA-grafted microbeads (250 μg) were mixed in 50 μL of 0.1% Tween 20 aqueous solution with 2 μL of 3×10^{-3} M polythiophene solution, and the mixture was stirred gently for 30 min at room temperature. The use of an excess of 2.4 equiv of polymer positive charges versus DNA negative charges was found to lead to optimal duplex formation at the bead surface. These "duplex microbeads" were then decanted and washed (once with 50 μL of 0.1% Tween 20 and twice with 50 μL of TT buffer) to remove any unbound polymer; then they were suspended in 50 μL of TT buffer and diluted 10 000-fold in TT to provide a ~30 beads/μL suspension. For the on-bead detection experiments, a 5 μL aliquot of this suspension was mixed in a quartz cell (Starna Cells, Inc., Atascadero, CA) with 3 mL of pure water at 65 °C to give ~150 beads in 3 mL or ~8 beads in the 150 μL volume probed by the custom-made fluorimeter.[14] The fluorescence from this solution, taken as the blank signal, remained stable during the entire duration of the measurements. A calibration curve and LOD were obtained by measuring the fluorescence after the addition to this initial solution (3005 μL) of successive aliquots of dilute (5×10^{-15} M) complementary ssDNA target solution, after a hybridization and equilibration period of 5 min.

Microfabrication of Microelectromagnetic Traps. The microelectromagnetic traps (μ-EMT) used in these experiments consist of planar micron-scale gold conductors supported on SiO_2 (500 nm)/Si wafers, based on a design described by C. S. Lee et al.[38] A conducting seed layer of Cr (10 nm as an adhesion layer) followed by an Au layer (100 nm) were deposited on a cleaned substrate using an electron-beam evaporator. Next, a thick positive photoresist (AZ-9260, Clariant Corporation, Somerville, NJ) was spun onto the wafer and an electroplating mold was patterned by UV exposure for the circular conductor and the contact pads. Before electroplating, a reactive ion etch descum process was applied to remove any remaining photoresist. The mold was next filled with 5 μm of electroplated gold (Technic Gold 25 ES, Technic Inc., Providence, RI) at a DC current density of ~2 mA/cm^2 and a temperature of 40 °C. After the electroplating was completed, the photoresist mold was stripped with acetone. The nonelectroplated area of the gold seed layer was sputter etched, and the chromium adhesion layer was selectively etched using a commercial solution (Chromium Etchant CR-4S, Cyantek Corporation Inc, Fremont, CA). Figure 3 shows an electromagnetic ring trap at this stage of the microfabrication process. Finally, to complete the microfabrication process of our device, a thin (20 μm) insulating layer of premixed poly(dimethylsiloxane) (PDMS) (Sylgard 184, Dow Corning, Midland, MI) was spun on top of the conducting gold wire. Each device was mounted on a printed hybrid-circuit carrier, wire-bonded via its related connection pads, and appropriately encapsulated using an Epoxy resin, thereby yielding a sensor ready to be connected to an electronic breadboard. The thickness (40 μm) of this insulating layer was optimized to control the magnetic field profile.[38] With this process, embedded conductors with large cross sections are achieved, which allows the application of a high current (up to 1 A), but the experiments were performed using a maximum DC current of 300 mA to limit Joule heating.

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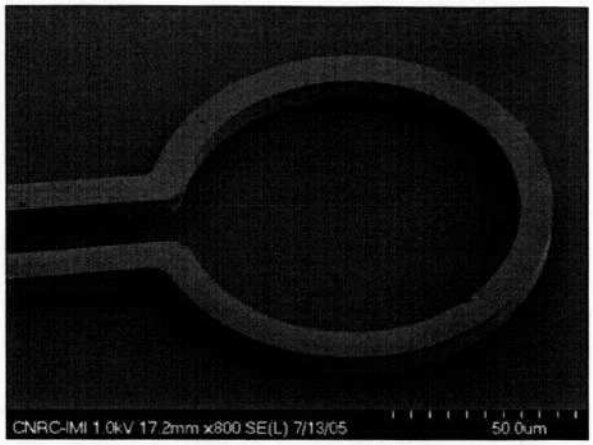

Figure 3. SEM picture of a microfabricated electromagnetic trap (without insulating layer).

Fluorescence Detection of Magnetically Confined Microbeads in μ-EMT. To evaluate the detection efficiency of the functionalized microbeads in the μ-EMTs, 3 μg of duplex microbeads were suspended in 70 μL of 0.1% Tween 20 and separated into 7 aliquots of 10 μL, each containing ~2.8×10^4 beads. Each aliquot was mixed with various amounts of target DNA, and the volume was completed to 50 μL with pure sterile water. The resulting mixture was incubated for 10 min at 65 °C and vortexed briefly, and then, a 25 μL droplet was deposited on top of the μ-EMT and covered by a glass coverslip (thickness ≈ 170 μm) and a ~1 mm spacer, which provided a flat optical surface and prevented water evaporation during the measurements. A 300 mA current was then applied to the μ-EMT for 5 min to attract and capture the microbeads; it was then lowered to 50 mA during signal acquisition to keep the beads in place while avoiding further trapping. The fluorescence signal was integrated over the entire inner surface of the μ-EMT, using a custom-made fluorescence detection system based on cw laser diode excitation at 408 nm, which overlaps well with the absorption spectral profile of the polymer transducer.[14] The laser beam was spatially filtered using a pair of plano-convex lenses and a 15 μm pinhole to obtain a near-Gaussian intensity profile. The laser beam was focused onto the sample with an infinity-corrected microscope objective (UPLFLN 10×/0.3 NA, Olympus America Inc., Melville, USA). Typical excitation power during the experiments was around 10 μW at sample level and was adjusted for an optimal S/N ratio. Fluorescence emitted from the sample was collected by the same objective. The collimated fluorescence light was steered toward the detector by a short wave pass dichroic beam splitter (~90% transmission at 408 nm, ~100% reflection at 530 nm, CVI Laser Corporation, Albuquerque, NM) through an interference filter of appropriate central wavelength and bandwidth (530/20 nm, Spectra Physics, Franklin, MA) to block light outside the emission band of the polymeric transducer. A plano-convex lens was then used to focus the collimated fluorescence onto a 50 μm pinhole located in front of a photon-counting PMT module (H7421-40, Hamamatsu, Bridgewater, NJ). Time-integrated pulse counts were transferred to a PC running a LabVIEW (National Instruments) user interface for data acquisition and analysis. The μ-EMT was positioned using a XYZ translation stage powered by DC servo

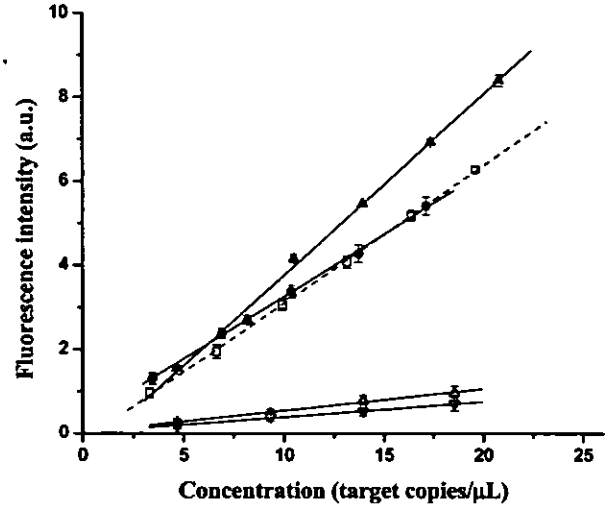

Figure 4. Calibration curves for dsDNA targets using the capture and release scheme: ▲, *C. albicans*, perfect match (polymer/X1:Y1); ●, *C. albicans*, perfect match (polymer/X1:Y1) with 10 equiv of excess of human genomic DNA; □ (- - -), *B. anthracis*, perfect match (polymer/X2:Y2); △, *C. albicans*, one mismatch (polymer/X1:W1); ▽, *C. albicans*, two mismatches (polymer/X1:W2).

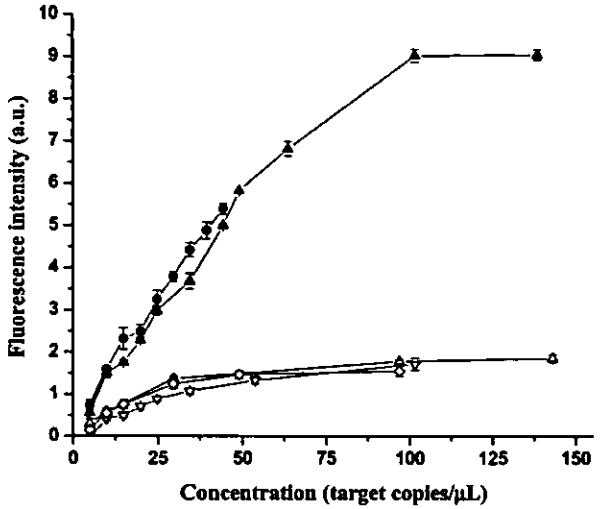

Figure 5. Calibration curves for dsDNA targets using on-bead detection scheme: ● and ▲, two experiments with *C. albicans*, perfect match (polymer/X1:Y1); △, *C. albicans*, one mismatch (polymer/X1:W1); ▽, *C. albicans*, two mismatches (polymer/X1:W2); ◇, *C. albicans*, fully mismatched (polymer/X1:X1).

actuators (Model Z612, Thorlabs, Newton, MA) and controlled in LabVIEW.

RESULTS AND DISCUSSION

As a basis for comparison of the analytical performances obtained with on-bead detection in the μ-EMT, we first characterized the use of the polymer transducer with magnetic beads in a "capture and release" scheme (Figure 2a). The target DNA present in a large sample volume was captured by functionalized magnetic microbeads, separated from the sample matrix, released in a smaller volume of a suitable aqueous media, and finally detected with the polymer–probe complex. The calibration curves obtained from these experiments are shown in Figure 4. The detection limits achieved using the polymer transducer and magnetic microbeads are 75 and 140 DNA target copies in the 150 μL probed sample volume (0.5 and 0.7 copy/μL) from the yeast *C. albicans* and the pathogen *B. anthracis*, respectively. This level of sensitivity is several orders of magnitude better than the previously reported method combining bead capture with detection by conjugated polymers.[33] In comparison with perfect hybridization, negative controls performed with double- or even single-mismatch targets induce only a very slight increase in fluorescence. Furthermore, the addition of a large excess (10 equiv) of nonspecific dsDNA to the perfectly matched *C. albicans* samples (6×10^4 target copies, 20-mer oligonucleotides), in the form of human genomic DNA (8×10^5 copies, up to 20 kbp in length), did not significantly affect the selectivity and sensitivity (LOD of 0.8 copy/μL) (Figure 4). These capture and release experiments were done with a preconcentration ratio of 100 (from 1 mL to 10 μL). However, considering the large number of probes available on each magnetic bead (2×10^6) and the fact that target molecules can be released quantitatively after capture, bead capacity is not expected to be a limiting factor, and larger preconcentration ratios (and correspondingly lower detection limits) could be achieved by this approach. Rather, the limiting factors in such cases would be the time needed for a target ssDNA molecule to be captured by a probe-grafted microbead and the time needed to magnetically capture beads dispersed in larger sample volumes, both of which could be minimized by implementing the above method in a compact microfluidic device with better mixing and capture dynamics.

A significant improvement in detection time can be achieved by using the polymeric transducer to detect the hybridization event directly on the magnetic beads. Successive additions of *C. albicans* Y1 target DNA to a suspension of duplex microbeads (~150 beads in a 3 mL cuvette or ~8 beads in the 150 μL probed volume) prepared with the complementary probe, X1, generated, within 5 min, a stable fluorescence signal directly proportional to the amount of added target DNA (Figure 5). The linear range of the calibration curve was found to reach ~100 DNA copies/μL. The curve deviation observed at higher concentrations could result from the increasing electrostatic and steric hindrance originating from the anionic triplex molecules (dsDNA + polymer) at the bead surface toward the negatively charged target ssDNA molecules. Negative controls performed using one, two, and fully mismatched targets show that the fluorescence signal increases very slightly until a concentration of 30 copies/μL and stabilizes afterward, with a minimum detection contrast (complementary vs noncomplementary) of 2.5 below 30 copies/μL and greater than 5 at higher concentrations. The detection limit of 1.4 target copies/μL (or 210 molecules in a probed volume of 150 μL), calculated from the linear portion of the calibration curve, is very similar to that obtained during the capture and release experiments. These results indicate that the polythiophene transducer is able to switch between its planar and twisted conformations in response to the hybridization event while complexed to DNA grafted on the bead surface, within the ordered and highly oriented layout of DNA probes attached to the surface via streptavidin–biotin couplings, and they suggest that this molecular structure could be used for the multitarget detection of DNA attached to solid substrates.

The benefits of on-bead DNA detection using the polythiophene transducer are best realized by measuring the fluores-

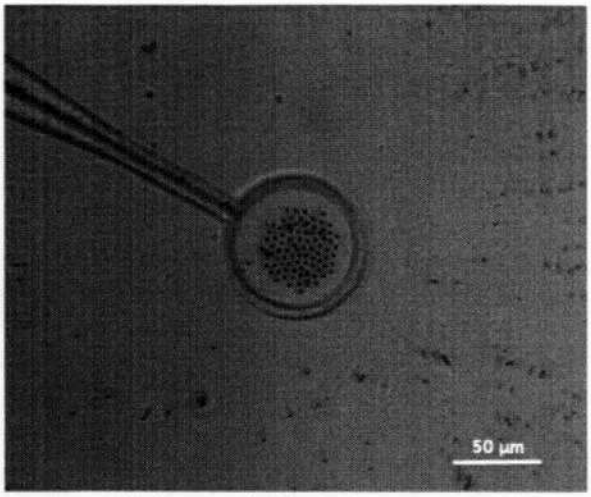

Figure 6. Optical image of microbeads trapped on μ-EMT.

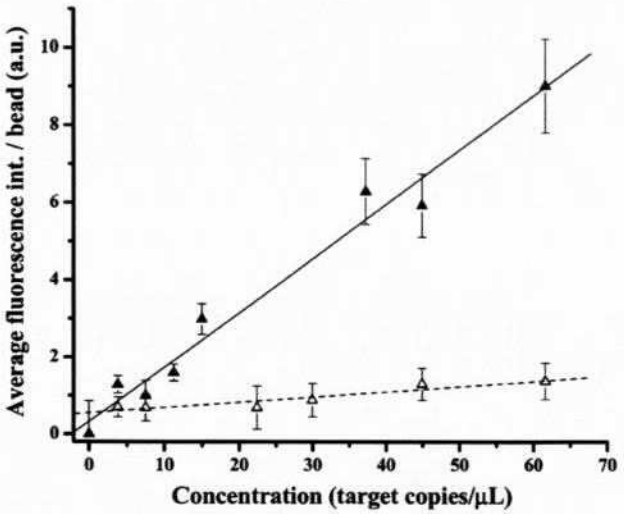

Figure 7. Calibration curves for dsDNA targets using on-bead detection in μ-EMT device: ▲, *C. albicans*, perfect match (polymer/X1:Y1); △, *C. albicans*, one mismatch (polymer/X1:W1).

cence from the microbeads while they are being magnetically confined in a small volume, which decreases the final sample volume and decreases the power requirements for the excitation source. In the present work, such a demonstration was performed in the static conditions prevalent in a solution droplet deposited on top of a μ-EMT. The selected experimental conditions led to the capture of a single layer of microbeads on the μ-EMT (Figure 6), which facilitated the interpretation of the fluorescence signal intensity. Visual observation of the trapping process under white light illumination revealed that duplex beads exhibit a lower mobility in the μ-EMT magnetic field than the triplex beads, possibly because of different surface charge densities and electrostatic interactions with the PDMS surface. While this behavior might eventually be exploited as a means to dynamically enhance the trapping of target-bearing microbeads, its effect on trapping efficiency and on the evaluation of detection limits was compensated for with the use of a constant concentration of duplex beads in the droplets and the variation of the number of DNA targets per bead. For example, the addition of 3.6 μL of a 10^{-12} M target ssDNA solution to a duplex bead aliquot (2.8×10^4 beads) resulted in 2.2×10^6 triplex molecules in 50 μL or ~80 triplex molecules/bead. The integrated fluorescence intensity measured within the loop was normalized to the number of trapped beads, and background subtraction was performed using nonhybridized duplex beads.

As shown in Figure 7, the integrated fluorescence intensity increases linearly with the concentration of perfectly matched DNA target, and the linear range of the calibration curve was found to extend to at least 60 copies/μL. Negative controls performed with ssDNA SNPs (single mismatch) resulted in a very slight increase in fluorescence with concentration and show that on-bead detection in the μ-EMT offers the same degree of selectivity as the capture and release experiments in homogeneous media. A detection limit of 7 copies/μL or 150 target molecules per bead was calculated from the calibration curve, which is somewhat higher than those achieved in homogeneous solutions using either capture and release or on-bead detection schemes. This difference is the result of the higher signal background caused by scattering of the excitation light from the multilayered μ-EMT structure, which has not been optimized with regards to its scattering and transmission properties, as well as from the beads themselves. Smaller beads, for example 200 nm diameter (d) beads (which are commercially available), would scatter excitation light to a lesser extent and would very likely result in a better signal-to-noise ratio. However, the magnetic force exerted on such beads would be appreciably reduced because it scales as $(d/2)^3$ and would thus require a longer trapping time or the design of a new μ-EMT geometry to generate a stronger magnetic field and achieve the same trapping efficiency as for larger beads.[39]

CONCLUSIONS

We have presented herein a new detection approach capable of performing ultrasensitive and sequence-specific sensing of minute amounts of DNA from large sample volumes, without any labeling or amplification of the nucleic acids, by combining the capture of DNA targets on functionalized magnetic particles with a fluorescent cationic polymer with the ability to optically transduce the hybridization of ssDNA targets with complementary ssDNA probes. We have shown how this approach can be used for the "capture and release" of target DNA, in which the targets are first captured on magnetic beads functionalized with suitable ssDNA probes, separated magnetically from the sample matrix, and released in a smaller volume of suitable aqueous media, where they are detected by polymer−probe ssDNA complexes. This approach was shown to offer detection limits in the zeptomolar range and the capacity to specifically detect low levels of target oligonucleotides in the presence of an excess of human genomic material. Interestingly, as long as two unique oligonucleotide sequences are known on the same DNA target, this target and release scheme could be performed with two different DNA probes in a double-recognition scheme (i.e., one for capture and one for detection) to provide even greater detection selectivity.

We have also demonstrated that the magnetic capture and detection steps can be combined on the same solid support by preparing polymer−probe ssDNA microbeads, where the polymer transducer is used to detect target DNA directly on magnetic

(39) Le Drogoff, B. et al. in preparation.

particles. This scheme increases sample throughput by eliminating the need to release the hybridized target DNA prior to optical detection and can yield results in only 5 min. Detection limits obtained with this scheme are very similar to those obtained during capture and release experiments, which indicates that the polythiophene transducer is able to switch between its planar and twisted conformations in response to DNA hybridization even while complexed to DNA grafted on the bead surface, in a fashion similar to the micellar polymer-based structures reported recently.[19,40] The fluorescence from these biosensing microbeads can also be read while they are being magnetically confined in the center of a microelectromagnetic trap (μ-EMT), which offers the possibility of increasing the signal-to-background ratio by simultaneously decreasing the final sample volume and detecting the fluorescence signal on the same support. Ultimately, such an approach could allow all the stages of a complex analytical procedure to be integrated on a chip, which would provide improved sensitivity, increased throughput, and decreased reagent consumption, as well as the possibility to perform point-of-care diagnostics and field analysis.

(40) Doré, K.; Leclerc, M.; Boudreau, D. *J. Fluoresc.* **2006**, in print.

ACKNOWLEDGMENT

This work was supported by the Chemical, Biological, Radiological and Nuclear Research and Technology Initiative (CRTI), Project 03-0005RD.

Received for review March 16, 2006. Accepted April 20, 2006.

AC060486N