ELECTROKINETICALLY INTEGRATED ISOLATION AND AMPLIFICATION OF PROTEIN-BINDING NUCLEIC ACIDS ON A MICROCHIP

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ABSTRACT

This paper presents a microchip that integrates the isolation and amplification of target-binding DNA strands in a randomized DNA mixture. Target-binding strands are isolated in the chip via binding with human immunoglobulin E (IgE) immobilized on microbeads, electrophoretically transported through a gel-filled microchannel, captured onto microbead-tethered reverse primers, and amplified using polymerase chain reaction (PCR). Integration of isolation and amplification is achieved using electrophoretic transport through the gel-filled microchannel, which prevents contamination of buffers or reactants while transferring the desired target-binding DNA. Experimental results show that our microchip can isolate and amplify target-binding DNA strands with increased binding affinity to IgE protein.

KEYWORDS

Microchip, electrophoresis, polymerase chain reaction, aptamer, affinity binding

INTRODUCTION

Analytes in biological samples are often present in minute quantities and contaminated with impurities in samples; it is thus of great interest to develop efficient methods and devices to isolate and enrich such analytes. In particular, isolation and amplification of DNA molecules have important applications to clinical detection of disease-related DNA markers [1] and synthetic selection of analyte-specific nucleic acids such as aptamers [2, 3]. Recently, microfluidic technology employing solid-phase extraction and electrophoretic separation has been applied to improve enrichment efficiency. However, existing microfluidic devices still typically require additional off-chip processes to isolate target-specific analytes from a solution. We recently developed a microchip in which target-binding DNA can be isolated and enriched by bead-based isolation and gel-based electrophoretic transport; however, the device was not yet capable of integrated DNA amplification, which is needed for assays such as clinical detection of DNA biomarkers [4] and binding affinity measurements [5].

This paper presents a microfluidic chip that fully integrates solid-phase-based DNA isolation with amplification of the isolated nucleic acids by polymerase chain reaction (PCR) using electrokinetically based molecular manipulation. In the chip, target-binding single-stranded DNA (ssDNA) is isolated by human IgE-functionalized microbeads in a chamber, electrophoretically transported through a gel-filled channel, and amplified on bead surfaces in another chamber. The gel physically separates microchambers on-chip, allowing desired ssDNA to be electrophoretically transported and eliminating cross-contamination. The combination of bead-based nucleic acid isolation, gel-based electrophoretic nucleic acid transport, and PCR simplifies microchip design, fabrication, and operation by eliminating the need for complex flow handling components. Experimental results show that our microchip can isolate and amplify IgE-binding ssDNA strands with increased binding affinity.

PRINCIPLE AND DESIGN

In our device, randomized ssDNA is incubated with IgE-functionalized beads in the isolation chamber (Figure 1a). Weakly bound ssDNA is then washed away and strongly bound strands are thermally eluted at 57°C (Figure 1b-1c). The eluted strands are electrophoretically transported into the PCR chamber where they are captured onto reverse-primer coated microbeads (Figure 1d-1f). The captured strands are amplified on the bead surfaces via PCR. The amplified strands are collected for binding affinity tests (Figure 1g).

The microchip consists of isolation and PCR amplification chambers (volume: 5 μL) having weir structures (depth: 40 μm) for trapping beads (diameter: 100 μm). Integrated resistive heaters and sensors (Cr/Au: 5/100 nm) control the chamber temperature during thermal elution and PCR amplification in the isolation and PCR chamber, respectively. The two chambers are connected by a channel filled with 3% agarose gel (7 mm × 0.8 mm × 40 μm). The additional channel lengths (0.4 μm) between the agarose-filled channel and each chamber provide thermal insulation to the agarose gel when the chambers are heated. These additional channels are filled with buffer through supplementary inlets. An electric field (25 V/cm) for DNA electrophoresis is generated by platinum electrodes that are inserted through bead inlets (Figure 2).

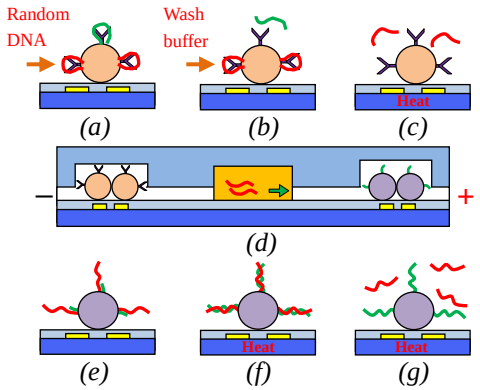

Figure 1: Illustration of ssDNA isolation and amplification using the microchip: (a) incubation, (b) wash, (c) elution, (d) electrophoretic transport, (e) hybridization, (f) PCR amplification, and (g) denaturation and release.

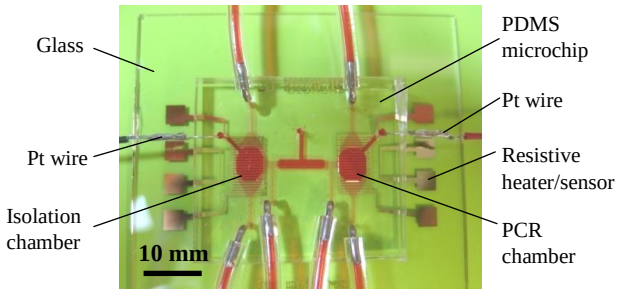

Figure 2: An image of the microchip. The chip is filled with red ink for visualization.

FABRICATION

The microchip was prepared using conventional microfabrication techniques. Briefly, layers of SU-8 photoresist were spin-coated on a clean silicon wafer and baked on a hotplate. To define the shape of the microchip, the solidified photoresist layers were exposed to UV light through photomasks and developed. Polydimethylsiloxane (PDMS) pre-polymer was spread onto the SU-8 mold, baked, and peeled off from the mold. Meanwhile, chrome and gold layers were deposited on a clean glass substrate and patterned to form the resistive heaters and sensors using positive photolithography. The heater/sensor layer was passivated with silicon dioxide (thickness: 1 μm) using plasma-enhanced chemical vapor deposition. After creating access holes in the PDMS slab, it was bonded on the glass substrate using oxygen plasma treatment. Molten agarose gel was injected into the channel through the gel inlet and allowed to solidify.

EXPERIMENTAL

Isolation and enrichment of target binding nucleic acids in a randomized ssDNA mixture was carried out as follows. The isolation chamber was filled with approximately 4×10^4 IgE-functionalized beads through a bead inlet using a syringe. The beads were then washed with PBS buffer modified with 1 mM $MgCl_2$ for 5 minutes at a flow rate of 40 μL/min using a syringe pump. The random ssDNA mixture was introduced to the chamber, incubated with the beads for 30 minutes, and collected from the outlet in tubes (~33 μL/tube). Weakly bound DNA strands were washed from the beads with PBS buffer (40 μL/min) while the waste solution was collected in separate tubes at the outlet (~33 μL/tube). The two chambers were filled with 0.5× TBE buffer containing 100 mM Na^+ and the isolation chamber was heated at 57°C for 5 minutes via the resistive heater to elute strongly bound strands. During the thermal elution, Pt electrodes were inserted into the chambers to generate an electric field of 25 V/cm. The DNA strands were then electrophoretically transported through the gel-filled channel and hybridized to the reverse primers immobilized on the beads in the PCR chamber. The captured strands were then PCR amplified on the beads by thermal cycling using the resistive heater. The amplified strands were separated from the complementary strands on beads by heating the chamber to 95°C and collected from the outlet. To perform the binding affinity measurement, ssDNA strands were incubated with approximately 5 μL of IgE-beads and eluted by heating at 95°C. The concentration of eluted DNA strands was then measured using a fluorescence spectrometer.

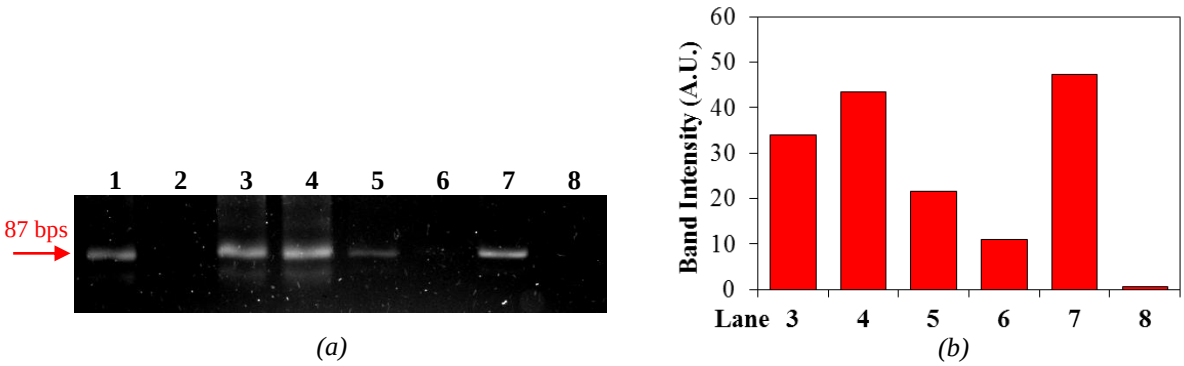

Figure 3: (a) Gel electropherogram of amplified eluents obtained from the isolation chamber. (b) Band intensity of each lane. Lanes 1: positive, 2: negative, 3: incubation, 4-6: washes, 7: elution, 8: wash from the PCR chamber.

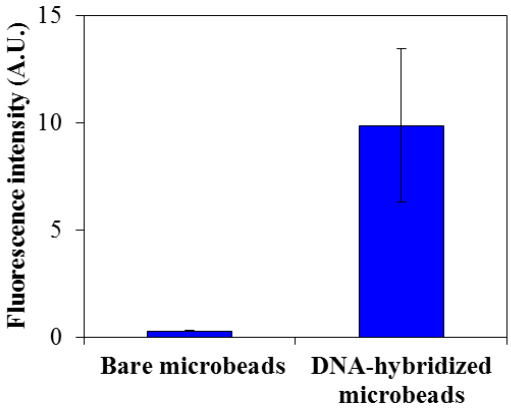

Figure 4: Changes in fluorescence intensity of primer-coated microbeads in the PCR chamber following capture of ssDNA.

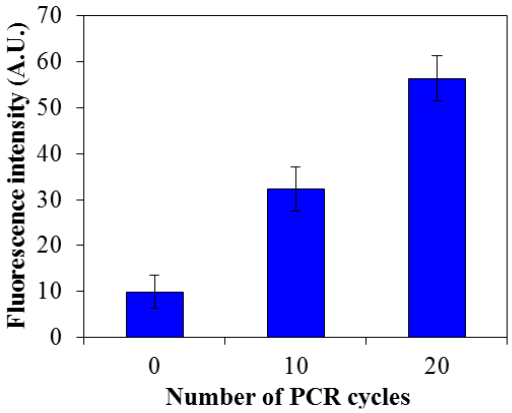

Figure 5: Changes in fluorescence intensity of DNA-hybridized beads in the PCR chamber as a function of the number of PCR cycles.

RESULTS AND DISCUSSION

To investigate the isolation of IgE-binding ssDNA, eluents from each step were amplified with PCR (14 cycles) and visualized using gel electrophoresis with intercalating dyes. DNA strands that did not bind to the beads during incubation are represented by the band in lane 3. The decrease in band intensity from lanes 4 to 6 indicates that weakly bound ssDNA were gradually removed from the beads during washing, while the bright band in lane 7 represents ssDNA that was strongly bound to IgE. No band in lane 8 indicates no DNA entered the PCR chamber during isolation of ssDNA (Figure 3).

We investigated the electrophoretic transport of DNA from the isolation chamber to the PCR chamber. The increase in the fluorescence intensity of beads in the PCR chamber indicates that the electrophoretically transported strands were hybridized to reverse primers that were immobilized on the beads (Figure 4). Then the captured DNA strands were amplified on the beads via thermal cycling using the integrated resistive heater. As the number of thermal cycles increased, a stronger fluorescent signal on the beads were observed indicating that the density of DNA on the beads increased (Figure 5). The resulting isolated ssDNA strands that were separated from the beads in the PCR chamber have been observed, via off-chip fluorescence measurements, to possess increased binding affinity over the random ssDNA strands to IgE protein (data not shown).

CONCLUSION

We have demonstrated an integrated microfluidic chip for isolating and amplifying ssDNA that bind to human IgE. In the chip, IgE-binding ssDNA strands are isolated by target-functionalized microbeads, electrophoretically transported through a gel-filled microchannel, captured via hybridization, and amplified on microbeads. Cross-contamination of buffers in different microchambers is eliminated with the gel-filled channel. Because complex flow handling components are not necessary, operation of the device is simplified. Experimental results show that DNA strands with increased binding affinity to IgE can be isolated and amplified in our microchip.

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