A MICROCHIP FOR NUCLEIC ACID ISOLATION AND ENRICHMENT

J. Kim1, J.P. Hilton1, K.A. Yang2, R. Pei3, K. Ennis4, M. Stojanovic5, and Q. Lin1

1Department of Mechanical Engineering, and
2Department of Medicine, Columbia University, New York, USA

ABSTRACT

This paper presents a microchip that effectively isolates and enriches target-binding single-stranded DNA (ssDNA) in a randomized DNA mixture using solid-phase extraction (SPE) and electrophoresis. The microchip consists of isolation and enrichment microchambers that are connected by a microchannel partially filled with agarose gel. Single-stranded DNA oligomers in the randomized mixture are captured via specific binding onto human immunoglobulin E (IgE) that is immobilized on microbeads in the isolation chamber. Following elution, the oligomers are separated from impurities via electrophoretic transport through the gel-filled microchannel to the enrichment chamber. Experimental results show that this approach can enhance the sensitivity of ssDNA detection methods in dilute and complex biological samples.

INTRODUCTION

In bioanalytical assays, analytes of interest in biological samples are often present in minute quantities and are contaminated with impurities. Thus, sample preparation steps prior to analysis are essential for improving the resolution of detection systems [1]. In particular, isolation and enrichment of DNA molecules within dilute and complex samples can enable clinical detection of disease-related DNA markers and synthetic selection of analyte-specific molecules such as aptamers.

Many techniques have been developed to address the need for sample pre-treatment by using microfluidic devices to separate and amplify low-concentration biological molecules from non-specific compounds in complex samples. Solid-phase extraction (SPE) and electrophoretic methods are commonly used within microfluidic devices for sample pre-concentration [2]. In a solid-phase extraction (SPE) device, target analyte is retained on a solid phase (e.g., microbeads) and impurities in the sample solution are discarded. Alternatively, electrophoresis can effectively concentrate charged molecules in a microchip.

Existing devices are limited by a lack of specific isolation of analytes from sample solutions. For example, SPE devices typically employ hydrophobic or electrostatic interactions between analytes and the solid phase, which are inherently nonspecific. Additionally, electrophoresis devices separate molecules based on mass-to-charge ratio, thus requiring additional processes to isolate target-specific analytes from a solution. Also, existing devices do not provide effective separation between enriched products and raw samples, contaminating the product solution.

To address these limitations, we have developed a microchip that effectively isolates and enriches IgE-binding single-stranded DNA (ssDNA) molecules using a combination of SPE and electrophoresis methods. The microchip consists of two microchambers for isolation and enrichment of target-binding DNA molecules. The two chambers are connected by a microchannel partially filled with agarose gel. In our device, ssDNA molecules are isolated via specific capture onto IgE-functionalized microbeads in the isolation chamber, and are concentrated in the enrichment chamber by electrophoretic transport. Experimental results show that this approach can enhance the sensitivity of ssDNA detection systems.

PRINCIPLE AND DESIGN

The isolation and enrichment processes for nucleic acids in a microfluidic chip are schematically shown in Figure 1. A random pool of nucleic acids is introduced to the target-functionalized microbeads (Figure 1a) placed in the isolation chamber. Following capture of ssDNA by the beads, wash buffer is flushed through the chamber to remove weakly bound ssDNA (Figure 1b). Strongly bound ssDNA is then eluted from the beads chemically using 0.1 M sodium hydroxide (NaOH) (Figure 1c). The eluted strands are transported to the enrichment chamber by applying an electric field across a channel partially filled with 3% agarose gel. The gel-filled channel effectively prevents the mixing of the different solutions in the two chambers while allowing the transport of isolated ssDNA molecules (Figure 1d). These steps are repeated to concentrate target-binding DNA molecules in the enrichment chamber.

The microchip consists of two 5 μL chambers (depth: 200 μm) for isolation and enrichment connected by a microchannel (1 mm × 7.8 mm × 40 μm) (Figure 2). Microbeads (diameter: 100 μm) are retained by a dam-like structure (weir, height: 40 μm) in the isolation chamber. The channel is partially filled with gel (3% agarose or 12% polyacrylamide) through an inlet. An additional length of channel (0.4 mm) thermally insulates the gel from the heated chambers when heating is used to elute the ssDNA from the beads. Supplementary inlets are used to fill these areas with buffer. Platinum (Pt)-wire electrodes are inserted through Pt inlets to provide a potential difference across the chambers for electrophoresis.

Figure 1: An illustration of isolation and enrichment of single-stranded DNA oligomers in a microchip.
Figure 2: (a) Schematic of the DNA isolation and enrichment microchip. (b) Photograph of the microchip (with the chambers and channels filled with red ink, and the gel between the chambers dyed blue).

EXPERIMENTAL

Device Fabrication

The microchip is fabricated using conventional microfabrication techniques such as lithography (Figure 3). Briefly, a silicon wafer is cleaned by soaking in sulfuric acid solution (Nanostrip, Cyantek Corporation) overnight. Layers of SU-8 photoresist are spin-coated on the silicon wafer and exposed to UV light through photomasks to define a mold for generating PDMS microchannels. Polydimethylsiloxane (PDMS) pre-polymer (Sylgard 184, Dow Corning) is then spread onto the SU-8 mold and baked at 75 °C for 1 hour on a hotplate. After punching access holes for inlets and outlets in the cured PDMS, it is bonded to a piranha-cleaned glass substrate following oxygen plasma treatment of the bonding surfaces. Inlet and outlet ports are connected to plastic tubes for sample handling. Three percent agarose gel in liquid state is injected to partially fill the microchannel through the gel inlet and is allowed to solidify.

Sample Preparation

Human myeloma IgE (Athens Research & Technology) is dissolved in 1× phosphate-buffered saline (PBS buffer) to a final concentration of 1 μM. NHS-activated microbeads are purchased from GE Healthcare and functionalized with IgE. An 87-mer ssDNA library having random sequences (5'-GCC TGT TGT GAG CCT CTC GAA-3') and fluorescently labeled IgE-specific ssDNA aptamer D17.4 (78-mer, K_D = 10 nM) 

Experimental Procedure

Isolation and enrichment of desired ssDNA molecules in a randomized ssDNA mixture is carried out as follows. The IgE-beads are loaded in the isolation chamber using a syringe through a bead inlet to fill approximately 30% of the chamber volume. After loading, the beads are washed for 5 minutes with PBS buffer at a flow rate of 20 μL/min using a syringe pump. Throughout the experiments, a DNA mixture having 0.1% of IgE-specific aptamer D17.4 is used for isolation of ssDNA with higher affinity to IgE, by increasing competition for IgE binding sites. The ssDNA mixture is introduced to the chamber and collected from the outlet in 3 separate samples (sample volume: ~33 μL). PBS buffer is introduced to the chamber at 20 μL/min to release weakly bound DNA strands from the IgE-beads, and the waste solution is collected in 10 separate samples at the outlet (sample volume: ~30 μL). To elute strongly bound DNA strands from the beads, 5 μL of 0.1M NaOH in 0.5× TB buffer is introduced into the chamber and incubated for 5 minutes.

Platinum electrodes are inserted into the Pt inlets in each chamber and an electric field of 25 V/cm is applied. The strands are then electrophoretically transported to the enrichment chamber through the gel-filled channel. Following electrophoretic DNA transport, the isolation chamber is washed with PBS buffer to remove undesired DNA molecules. To concentrate the IgE-binding ssDNA in the enrichment chamber, the isolation and electrophoretic transport processes are repeated.

Samples of eluent containing ssDNA molecules are collected throughout the experiment and amplified using polymerase chain reaction (PCR). Slab-gel electrophoresis is used to evaluate the concentration of DNA eluted throughout the process by comparing the band intensities for each sample. A fluorescence microscope is used to monitor the electrophoretic transport of ssDNA through the gel-filled channel.
RESULTS AND DISCUSSION

We first investigated the isolation of IgE-binding ssDNA from the randomized ssDNA mixture in the isolation chamber. Figure 4(a) shows a gel electropherogram of the PCR products of eluents collected during the isolation experiment. In the image, 87-bp bands represent the random ssDNA in the pool while 78-bp bands represent D17.4 aptamer. Lanes 1 and 2 are positive (a mixture of random ssDNA and D17.4 aptamer) and negative (whole PCR mixture except DNA template) controls, respectively. The two distinct bands seen in lane 1 are for a PCR product of a mixture of random ssDNA containing D17.4 aptamer. No bands are seen in lane 2 indicating that reagents used during the experiment were not contaminated by undesired DNA molecules.

The band intensity profile of the 87-mer ssDNA during incubation, consecutive washes, and elution is shown in Figure 4(b). 87-mer ssDNA that did not bind to the IgE-beads during the incubation step are indicated by the bright band in lane 3. The decreasing intensity of the bands from lane 4 to lane 6 indicates that as washing continued, loosely bound 87-mer ssDNA molecules were removed from the bead surfaces, increasing the stringency of the isolation. Strongly bound 87-mer ssDNA molecules were eluted from the IgE-bead surfaces by 0.1M NaOH as shown in lane 7.

The enrichment chamber was washed with buffer just prior to electrophoretic migration of isolated DNA. To determine if the gel-filled channel prevents contamination of the enrichment chamber with unwanted DNA from the isolation chamber, the buffer was PCR amplified and analyzed using gel electrophoresis (Figure 4(a), lane 8). As no band is shown in that lane, the gel effectively blocked the unwanted ssDNA from contaminating the enrichment chamber.

Figure 4: (a) Gel electropherogram of eluents obtained during the isolation experiment. (b) Band intensity profile for incubation, washes, and elution samples.

To verify that the eluted 87-mer ssDNA is IgE-specific, we repeated the isolation experiment using fresh NHS beads without functionalizing the surfaces. Following all buffer washes, samples of buffer were PCR amplified using the same conditions as with the previous test (Figure 5). The gel image and band profile show bright bands for incubation (lane 3) and wash 1 (lane 4) while no bands are observed in washes 5 and 10 (lanes 5 and 6), and elution (lane 7). This indicates that ssDNA were bound on the NHS-beads very weakly and released during earlier washing steps. Hence, the 87-mer ssDNA collected in the previous experiment shown in Figure 4 are IgE-binding ssDNA isolated from the random mixture.

Figure 5: (a) Gel electropherogram of eluents obtained during the control experiment. (b) Band intensity profile for incubation, washes, and elution samples.

Figure 6: Electrophoretic transport of fluorescently labeled ssDNA through the gel under an electric field of 25 V/cm at different times: (a) t=0 min, (b) t=10 min, and (c) t=20 min. (d) Fluorescence intensity as a function of time monitored at the detection site.
We then investigated the electrophoretic transport of isolated ssDNA through the gel-filled microchannel. Two Pt electrodes connected to a DC power supply were inserted in the Pt inlets of the isolation and enrichment chambers to form a cathode and an anode, respectively. The electrophoretic transport of the ssDNA through the gel-filled channel was monitored using an inverted epifluorescence microscope. The fluorescence micrographs obtained at different times at the center in the channel are shown in Figures 6(a), (b), and (c). The peak in the fluorescence intensity profile at 10 minutes indicates that the eluted ssDNA were migrating at a speed of approximately 1 mm/min through the gel-filled channel (Figure 6d). As the distance between the two chambers is 20 mm, it took approximately 20 minutes to transport the ssDNA to the enrichment chamber.

Following the electrophoretic transport of IgE-binding ssDNA for 20 minutes, the two chambers were thoroughly washed with PBS buffer and the waste solutions were collected. A gel electropherogram of the collected eluents is shown in Figure 7. Bands in lanes 3, 4, and 5 correspond to ssDNA that did not bind to the IgE-beads during incubation. The band intensities in lanes 6-10 decrease due to continuous washing. The bright band in lane 11 corresponds for the electrophoretically transported ssDNA to the enrichment chamber. On the other hand, a very weak band is shown in lane 14 representing waste collected from the isolation chamber after electrophoresis. This indicates that the majority of the captured ssDNA have migrated from the isolation chamber to the enrichment chamber.

To investigate the ability of the chip to enrich DNA, multiple rounds of ssDNA isolation and transport were performed on a single chip. Following a round of isolation and transport, fresh IgE-beads were introduced to the isolation chamber. The captured ssDNA was then concentrated in the enrichment chamber by repetitive isolation and transport. A gel electropherogram for eluents collected after 1 round (lane 3), 2 rounds (lane 4), and 3 rounds (lane 5) of enrichment in different chips is shown in Figure 8(a). As expected, the band intensity profile shows an increase in band intensities as the number of enrichment rounds increases, indicating a higher concentration of DNA following a greater number of enrichments (Figure 8b).

CONCLUSION
We have presented a microfluidic chip that isolates and enriches ssDNA molecules from a randomized pool using IgE-functionalized microbeads. The developed microchip consists of two chambers (i.e., isolation and enrichment chambers) connected by a microchannel partially filled with agarose gel. The ssDNA oligomers are captured by IgE-functionalized beads in the isolation chamber, electrophoretically transported through the channel, and concentrated in the enrichment chamber. Multiple rounds of isolation and transport enrich IgE-binding ssDNA prior to detection and further analysis. The gel-filled channel allows electrophoretic transport of ssDNA while separating the enriched ssDNA from impurities in the isolation chamber. Thus, this microchip holds the promise for enhanced detection sensitivity of biological molecules such as ssDNA from dilute and complex biological samples.

ACKNOWLEDGEMENTS
We gratefully acknowledge financial support from the National Science Foundation (Award No. CBET-0854030) and the National Institutes of Health (Award Nos. RR025816-02 and CA147925-01).

REFERENCES

CONTACT
*J. Kim, tel: +1-212-8543221; jk3185@columbia.edu