Self-contained, Integrated Biochip System for Sample-to-Answer Genetic Assays

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Abstract

Microfluidics-based biochip devices are developed to perform DNA analysis from complex biological sample solutions. Microfluidic mixers, valves, pumps, channels, chambers, heaters, and DNA microarray sensor are integrated to perform magnetic bead-based rare cell capture, cell preconcentration and purification, cell lysis, polymerase chain reaction, DNA hybridization and electrochemical detection in a single, fully automated biochip device. Pathogenic bacteria detection and single-nucleotide polymorphism analysis directly from blood are demonstrated. The device with capability of on-chip sample preparation and DNA detection provides a cost-effective solution to direct sample-to-answer genetic analysis, and thus has potential impact in the fields of point-of-care genetic analysis and disease diagnosis.

Keywords: Sample prep, microvalve, micromixer, biochip

1. Introduction

A fully integrated biochip needs to perform all analytical functions including sample preparation, mixing steps, chemical reactions, and detection in an integrated microfluidic circuit. For most current biochips, sample preparation from the 'real' samples of bodily fluids represents the most difficult procedure to be realized in the chip level.

While most of the work has been directed towards the integration of PCR with CE, only few examples of combinations of sample preparation with PCR and DNA microarray have been described [1], [2]. Although good performance has been demonstrated, lack of efficient on-chip mixing, valving, and pumping techniques and the use of optical-based microarray detection make the above systems less desirable for some applications such as point-of-care diagnosis.

We report the development of a self-contained and fully integrated biochip system for sample-to-answer DNA analysis that starts from sample preparation (i.e., magnetic bead-based target cell capture, cell preconcentration and purification, and cell lysis), followed by PCR and microarray electrochemical-based detection. Complex biological sample and reagent solutions are placed on the device, while electrochemical signals corresponding to genetic information are the primary output.

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2. Design

The device (Fig.1) consists of a plastic fluidic chip, a printed circuit board (PCB), and a Motorola eSensor™ microarray chip. The plastic chip includes a mixing unit for rare cell capture, a cell preconcentration/purification/lysis/PCR unit, and a DNA micro-array sensor. The chip measures 60 x 100 x 2 mm and has channels and chambers that range from 0.5 to 1.2 mm in depth and 1 to 5 mm in width. The PCB consists of embedded resistive heaters and control circuitry. Motorola eSensor™ is a PCB substrate with 4x4 Au electrodes on which single-strand DNA probes are immobilized to detect electrochemical signals of hybridized target DNA. A portable instrument is built to provide power supply, PCR thermal cycling, and DNA electrochemical signal readout.

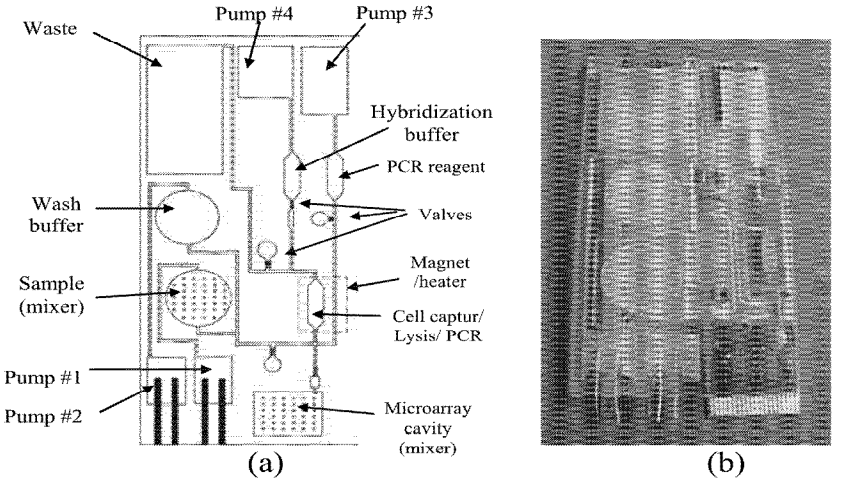

Fig. 1. (a) Schematic of the plastic fluidic chip. (b) Photograph of the integrated device that consists of a plastic fluidic chip, a large printed circuit board (PCB), and a Motorola eSensor™.

3. Experimental

Pipettes are used to place a biological sample solution (up to 1 mL) followed by a solution containing immunomagnetic beads in the sample storage chamber. Other solutions including a wash buffer, PCR reagents, and hybridization buffer are separately placed in corresponding storage chambers. Acoustic mixing based on cavitation microstreaming principle [3] is implemented in the sample storage chamber to ensure a fully mixing between the target cells and the immuno-magnetic capture beads. An electrochemical micropump based on electrolysis of a salty solution is used to transfer the sample mixture through the preconcentration/PCR chamber, where target cell capture and preconcentration occur as the bead conjugates are trapped by the magnet underneath the chamber, while the rest of the sample solution passes through to the waste chamber. The washing buffer is subsequently pumped through the preconcentration/PCR chamber to wash out the contaminants

and purify the captured cells. The solution containing PCR reagents is then moved into the preconcentration/PCR chamber. Subsequently, all the thermally-actuated paraffin-based microvalves [4] connected to the chamber are turned to "closed" positions followed by thermal cell lysis and PCR (30 thermal cycles). After PCR and the openning of the valves, the hybridization buffer and the amplification products are pumped into the detection chamber. A mixing process facilitated by cavitation microstreaming follows to ensure homogenous fluidic mixing and improve hybridization kinetics [3]. After hybridization, the electrochemical signals from ferrocene-labeled signal probes are detected and processed by the instrument. The complexity of the chip design is minimized by using some of the chambers for more than one function (e.g., the chamber to capture and preconcentrate target cells is also used for subsequent cell lysis and PCR) and performing PCR in presence of magnetic beads. These also result in less sample lost.

4. Results and discussion

Pathogenic bacteria detection from whole blood sample is performed in the device. The input sample is 1 mL of whole blood containing 10^3 to 10^6 E.coli K12 cells. 10 μL of biotinylated rabbit anti-E.coli antibody and 20 μL of streptavidin labeled Dynalbeads are also added into the sample storage chamber. The bead-antibody-E.coli cells complexes are formed during a 20-min acoustic mixing and incubation period. Following the cell preconcentration and purification steps, the purified bead-antibody-cells complexes are isolated from the blood sample solution and trapped in the PCR chamber. After the PCR reagents (20 μL) are transferred to the PCR chamber, thermal lysis followed by PCR is performed to amplify E.coli K12-specific gene fragment in the presence of beads. The PCR products are then transferred to the microarray chamber along with a hybridization buffer (20 μL). The electrochemical measurement of E.coli K12-specific gene is obtained (Fig. 2a). The typical cell capture efficiency using Dynalbeads in our experiments is about 40%. In the presence of 1.3×10^7 (20 μL) Dynalbeads, the amplification efficiency is reduced by 50% as compared to the control PCR reaction performed without Dynalbeads present. It is believed that the chip assay sensitivity can be improved by device and assay optimization.

High abundance DNA detection directly from blood sample is also demonstrated in the device. In this assay, the rare target capture and preconcentration steps are skipped since the PCR amplification can be directly performed on diluted blood samples. A mixture (20 μL) of a diluted human blood (1.4 μL) and PCR reagents is transferred to the PCR chamber. After the close of the valves, thermal cell lysis followed by a multiplexed asymmetric PCR amplification is performed to simultaneously amplify DNA sequences containing the sites for HFE-H and HFE-C polymorphisms. After the PCR is complete, the hybridization buffer and the amplification product are pumped to the microarray chamber. Genotyping results are shown in Fig. 2b. The whole analysis took 2.5 hr.

5. Conclusions

We have developed an integrated microfluidic biochip that can perform sample preparation and DNA analysis in a single device. Plastic chip fabrication and standard PCB process, coupled with the simple and inexpensive microfluidic techniques (including on-chip micromixing, valves, and pumps), are ideal for low-cost productions of this integrated device. This platform provides a potential solution for genetic analysis of complex biological fluidic samples in the fields of point-of-care genetic analysis and disease diagnosis.

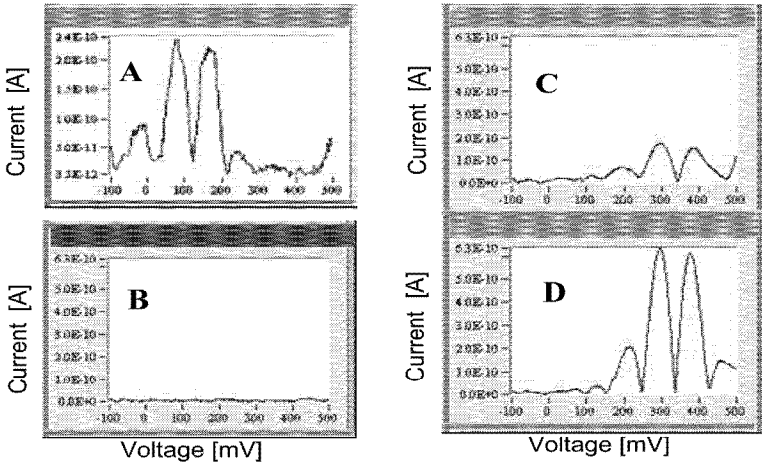

Fig. 2. Electrochemical measurement results: (**A**) Detection of 10^6 *E. coli* K12 cells from 1 mL of rabbit blood. (**B - D**) Genotyping results from 1.4 μL of human blood: (**B**) Negative control; (**C**) Identification of HFE-H gene; (**D**) Identification of HFE-C gene.

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References

[1] R. C. Anderson, X. Su, G. J. Bogdan, and J. Fenton, "A Miniature Integrated Device for Automated Multistep Genetic Assays," *Nucleic Acid Research*, vol. 28 (12), pp. e60: i-vi, 2000.

[2] P. K. Yuen, L. J. Kricka, P. Fortina, N. J. Panaro, T. Sakazume, and P. Wilding, "Microchip Module for Blood Sample Preparation and Nucleic Acid Amplification Reactions," *Genome Research*, vol. 11, pp. 405-412, 2001.

[3] R. H. Liu, R. Lenigk, J. Yang, R. Druyor-Sanchez, and P. Grodzinski, "Hybridization Enhancement Using Cavitation Microstreaming," *Analytical Chemistry*, vol. 75, pp. 1911-1917, 2003.

[4] R. H. Liu, J. Bonanno, R. Stevens, and P. Grodzinski, "Thermally Actuated Paraffin Microvalves," *Proc. Micro Total Analysis Systems 2002, Kluwer Academic Publisher, Dordrecht, The Netherlands*, pp. 163-165, 2002.