Magnetic bead handling in a PCR-CE microchip

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Introduction

Microchip polymerace chain reaction (PCR) offers fast DNA amplification with micro litre scale samples. Capillary electrophoresis (CE) in the same microchip makes possible to indentify amplified products. Microchip PCR-CE chips has been used for example to gene expression analysis [1], where affinity capture plug purifies and concentrates sample products. We have previously demonstrated both PCR and CE analysis separately in chip level [2,3]. In this paper we present the integrated PCR-CE chip with controlled magnetic beads handling. Beads can be used for sample concentration and preparation before the analysis.

Chip structure

PCR-CE microchip (Fig. 1) consists of a microfluidic silicon chip covered with a glass lid. The chip size is 7 x 37 mm^2. Silicon chip has fluidic structures for microbead preparation, PCR and CE. CE channel length is 33 mm and its width/depth is 75/75 µm. The depth of the PCR well is 375 µm and its volume is 1 µl. Pillar arrays (Fig. 2) have been added into the well for trapping bubbles or filtering microbeads. Pillar filters consist of 50 x 50 μm^2 pillars in three lines with 50 µm pillar spacing. Molybdenum thin film heating elements are integrated to the backside of the chip to tune the temperature of the PCR well.

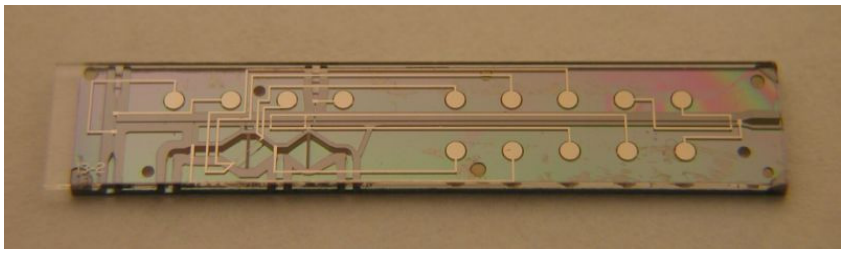

Fig. 1. Fabricated PCR-CE device. Liquid interface is realized by edge-coupling capillaries into the fluidic channels.

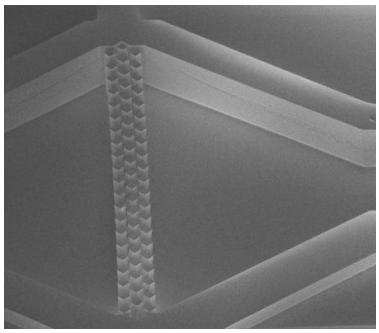

Fig. 2. Bubble filter with 50 x 50 µm silicon pillars.

Glass lid has platinum thin film elements for temperature measurement, high voltage contacts and conductivity detection. They locate between the silicon chip and the glass lid. Electrical interfacing to the chip is realized through the silicon substrate. Thermal oxide is used as surface passivation for silicon. It also provides a good insulation for CE. PECVD oxide is used to passivate thin film wirings on glass lid. Silicon chip and the glass lid are attached to each other by glue bonding. All these choices were tested for their surface chemistry with the method described in [4].

Measurements

Our measurement system consists of temperature controlling and fluorescence/conductivity detection. Temperature measurement setup is controlled with a laptop PC via an AD/DA driver card. The driver card measures the thermistor temperature and controls the heating power of the heating element. Fluorescence measurement setup is controlled with another PC with commercial program (Hamamatsu Wasabi) and LabView software. Both PCR amplification and CE separation

are monitored with Laser Induced Fluorescence (LIF) detector. There is also two channel conductivity detection in the separation channel. LIF detector has Argon laser (488 nm, 40 mW) for fluorescence excitation and cooled CCD camera for detection of fluorescence emission.

Magnetic beads are handled with a magnetic rod located outside the chip. Magnetic beads can be moved between microfluidic wells. They can be kept in one well when liquid is changed in the other. Pillar structures within the wells are used for bead washing process to prevent bead clustering. Bead cluster is broken with magnetic rod movement over pillars.

Bubble filter operation was tested successfully (Fig. 3). Air bubbles emerging in liquid input are captured to the silicon pillars of bubble filter. Magnetic bead handling was also tested. Beads were feeded with peristaltic pump in water like buffer solution. Pump velocity was about 1 µl/s. Magnetic rod captures magnetic beads (Ø=1 µm) in a well (Fig. 4). Beads were moved from one well to another and back. Beads followed magnetic rod also trough pillar filter.

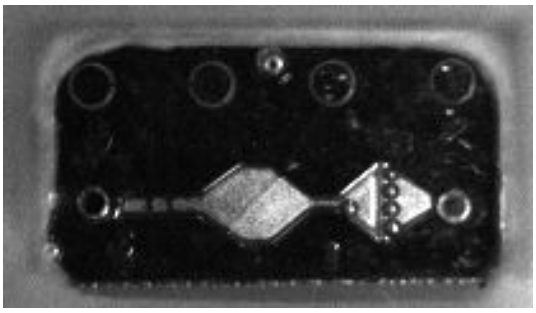

Fig. 3. Air bubble filtering.

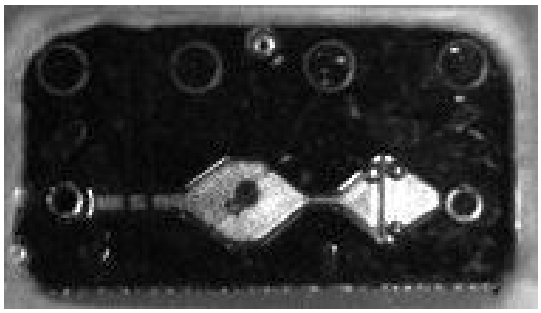

Fig. 4. Magnetic bead capturing

Conclusions

We have developed PCR and CE on the same microchip. PCR reaction cavities makes possible to do sample preparation and filtering. Magnetic beads were feeded in liquid flow and captured with external magnet. PCR cavities has bead size based pillar filter with 50 µm spacing. Magnetic beads (Ø=1 µm) goes trough pillars, but captured magnetic beads were moved trough pillars to prevent clustering. We were used pillars also for air bubble filtering. Our aim in the future is to use this magnetic bead based system to microchip PCR-CE analysis.

References

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Poster