Magnetic beads-based nucleic acids extraction in microfluidic chip

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Abstract. The dominant trend in molecular genetic methods research of biological samples is the automation of all stages, the most valuable of which is preparation of the sample. The use of microfluidic technologies provides not only full control of all operations at this stage, but also reduces the impact of the environment on the sample preparation process. Magnetic beads technology is one of the methods for extracting RNA and DNA with subsequent analysis. The paper presents magnetic beads-based nucleic acids extraction in the microfluidic chip via automatic mode with further registration by real-time polymerase chain reaction. We have shown that efficiency of DNA extraction in microchip is compared to that in case of conventional manual extraction and allows further improvement by means of automation.

1. Introduction

A significant number of infectious diseases, contamination of food and environment, genetic biomarkers of tumors and drug metabolism can be analyzed based on detection of nucleic acids [1]. Typical commercial kits for DNA extraction require many manual steps involving different instrumentation and sample handling stages for each process, which causes a cross contamination or loss of sample during transfer [2]. Integrated microfluidic platforms offer a unique solution to many of problems currently encountered in genetic analysis [3]. Microfluidic systems have various advantages compared with their large-scale counterparts, including low cost, disposability, low reagent and sample consumption and possibility for automation to be implemented [4].

Magnetic beads technology is widely used for capturing and manipulating of nucleic acids inside a microfluidic chip (MFC). The technique constitutes a separation of nucleic acids from complex mixtures via complementary hybridization [5]. In recent years, a great progress has been achieved in coupling of functionalized magnetic beads to suitable buffers systems which allows for a rapid and efficient extraction procedure [6]. Centrifugation steps used in traditional methods and causing the break of nucleic acids are avoided in magnetic beads technology, which allows maintaining intact longer fragments from genomic DNA [7]. Another favorable aspect of this technique is that it also gives an alternative way for automation of extraction procedures from a large number of samples [8].

In this work we demonstrate the possibility of magnetic beads-based nucleic acids extraction from a biological sample in microfluidic chip by automatic mode. A rapid and automated preparation of sample was performed with a polydimethylsiloxane (PDMS) chip, inside which probe-conjugated magnetic beads hybridize cDNA fragment of GAPDH gene released from lysed cell. A permanent magnet was used to manipulate complexes of magnetic beads and cDNA. The following
washing/purification process was carried out using a syringe pump. Subsequent registration by real-time polymerase chain reaction (PCR-RT) was observed.

2. Materials and methods

The MFC was fabricated from PDMS by soft lithography technique. Thoroughly mixed and degassed liquid PDMS mixture (Sylgard 184, Dow Corning) consisting of 10:1 silicon elastomer and a curing agent was poured onto the master mold and allowed to solidify at 80°C for 2 h. After complete curing, PDMS was peeled off from the mold and cut into pieces containing individual fluidic channel design. Inlet and outlet access holes were made with a biopsy punch [9]. Sealing was achieved by simply placing the PDMS replica onto a clean glass slide after surface oxidizing in O₂ plasma. Mold was fabricated using standard lithography from SU-8 negative photoresist on a silicon wafer [10].

Input, stirring and removing of liquid in a microchip reaction chamber were carried out using both a syringe pump Harvard PHD 2000 (USA), controlled by LabVIEW real-time interface system, and a system of supply silicone capillaries with an internal diameter of 1.15 mm.

The set of reagents "M-Sorb-OOM" (CJSC Sintol, Russia) intended for magnetic beads-based DNA/RNA extraction from clinical samples and objects of environment was used for experiments. A biological sample with nucleic acids was simulated by a model solution containing particular concentration of the cDNA fragment of GAPDH gene. After DNA extraction nucleic acids were analyzed by PCR-RT on ANK-32 device (IAI RAS, Russia). A set of specific primers with samples (DNA Synthesis, Russia) and reaction mixture M-428 "PCR-Mix" (CJSC Sintol, Russia) were used for the experiments. As a control sample a solution of nucleic acids was considered by traditional method in polypropylene test tubes after DNA extraction.

3. Experiment

A lysis of the model sample with a cDNA solution of GAPDH gene was carried out according to the manufacturer's instruction: a mixture of the sample, a lysing solution and a lysing component in polypropylene test tubes were incubated for 15 minutes at 65°C. Obtained lysate solution was divided into two equal parts to extract nucleic acids in MFC and in polypropylene test tubes respectively. Magnetic beads and precipitating solution for nucleic acid sorption were added to each part of the lysate solution.

The mixture of lysate with magnetic beads was pumped several times through the MFC reaction chamber at a flow rate of 50÷100 µl/min. By external permanent magnet, located under the MFC, magnetic beads with nucleic acids were retained in the reaction chamber. After removal of the solution from microchip magnetic beads with the sorbed cDNA were washed from the components of the lysing solution as it is shown on the scheme in figure 1. Each of three washing solutions was sequentially pumped through the reaction chamber three times in both directions by an automatic mode. Meanwhile the movement of the external magnet ensured a stirring of magnetic beads inside the MFC. Then the reaction chamber with magnetic beads was purged by air for 5 minutes to remove the washing solutions residues. For cDNA desorption 50 µl of eluting solution was repeatedly (ten times) pumped through the reaction chamber with magnetic beads. Afterward the filled microchip was incubated at 65°C for 10 minutes. The solution with eluted cDNA was collected in a test tube.

The second part of the solution with lysate was used to extract DNA in polypropylene test tubes. For magnetic beads washing and DNA desorption the same volumes of liquids were taken as for extraction in MFC. After inputting of the next solution, test tubes contents were mixed on a micro-centrifuge-shaker to uniform distribution of magnetic beads and incubated for a short time at room temperature. Afterward the test tubes were centrifuged and then supernatant was removed. To dry washing solution residues opened test tubes were incubated for 5 minutes at 65 °C. Then 50 µl of the eluting solution was added, mixed and kept for 10 minutes at 65 °C. The supernatant with cDNA was collected in a separate test tube.
Figure 1. Scheme of magnetic beads-based nucleic acids extraction in MFC.

4. Results
Volume of 2 µl of the eluted cDNA solution was added to the finished reaction mixture for PCR. Distilled water was used as a negative control. Figure 2 shows the kinetic dependences of PCR-RT amplification for samples obtained after nucleic acid extraction in the microchip and in the test tube, as well as for the initial model cDNA of GAPDH gene. The value of threshold cycle (Ct) was determined using software in the ANK-32 thrice. The Ct value is the cycle number which is needed for the PCR product fluorescence reaches threshold level above the background signal. In order to calculate the Ct, horizontal line (threshold) is drawn on the amplification plot. The placement of this line is determined by the software. Ct value is the point of crossing the curve and threshold level.

Table 1. Average value of the threshold PCR cycle for each analysed solution.

| Sample                               | Ct              | n=3          |
|--------------------------------------|-----------------|--------------|
| Extraction DNA on MFC                | 23.7±0.1        |              |
| Extraction DNA in test tube          | 23.5±0.1        |              |
| Initial concentration                | 21.9±0.1        |              |
According to experimental results of PCR-RT the efficiency of nucleic acids extraction in the microfluidic chip compared to initial concentration is $\alpha \approx 66\%$, and in the same time in the test tube $\alpha \approx 73\%$, respectively. Thus, the efficiency of magnetic beads-based nucleic acid extraction from the lysate of the sample in the MFC is comparable with the results of cDNA extraction in test tubes.

The advantage of using a microchip is the possibility of automation by the programmable pump and low reagent consumption comparing with traditional method. Also magnetic beads-based technology using MFC avoids centrifugation step which allows keeping DNA fragments unbroken.

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