

Fabrication of Amino Silane Coated Microchip for DNA Extraction from Whole Blood

T. Nagakagawa, T. Tanaka, H. Takeyama,
and T. Matsunaga

Tokyo University of Agriculture & Technology,
2-24-16 Naka-cho, Koganei-city, Tokyo, 184-8588, Japan

Lab-on-a-chip technology has been recently attracting much attention due to their increasing various applications. To design total analysis system on a chip, DNA extraction step plays an important role in subsequent reaction. On-chip DNA extraction has been mainly performed by DNA adsorption to silica in the presence of chaotropic salt. Recently, various micro fabrications of silica have been developed in order to enhance the efficiency of DNA trapping on a chip [1][2]. However, these microfabrications involve labor-intensive and time-consuming procedures. More recently, a novel DNA extraction method has been proposed by our research group [3]. The principle is based on electrostatic interaction between amine groups and nucleic acids. In this study, amine-coated microchips for DNA extraction were designed and fabricated. The binding ability of the microchip was examined by using λ -DNA. Furthermore, DNA extraction from whole blood on the microchip and subsequent PCR using recovered DNA was examined.

Microchip consisted of silicon wafer substrate with flow pass and a lid made of PDMS. Silicon wafers were used as substrates to fabricate a flow pass (Fig.1). To introduce amino groups on the surface, the microchip was incubated with 1% solution of amino silanes in toluene. λ -DNA (2 μ g/ml; total amount = 10 ng) in TE buffer (10 mM Tris-HCl, 1mM EDTA, pH7.5) was injected in amine coated microchip and incubated for 10 min. Then the flow pass on the microchip was rinsed three times with washing buffer (pH7.5). DNA samples trapped in the microchip were eluted with elution buffer (0.05 M sodium carbonate, 0.05 M sodium bicarbonate, pH10.6). DNA in the each fraction was quantified using Pico Green, which is an intercalator to recognize double stranded DNA. Furthermore, DNA extraction from whole blood was performed using blood lysate. Whole blood (5 μ l) was lysed with a buffer containing 50 mg/20 μ l proteinase K and 10% Triton-X100. After incubation at 55°C for 25 minutes, 5 μ l of the lysate was injected on the microchip.

The amount of DNA captured in the microchip increased with increasing incubation time. Amounts of DNA capture in and eluted from the microchip were consistent with the numbers of amine groups. Furthermore, DNA extraction from whole blood using amine-coated microchip was examined. The amount of DNA and protein in each fraction was quantified. Proteins were removed at washing steps and DNA was eluted by changing pH conditions (7.5 to 10.6). The amount of extracted DNA from 5 μ l whole blood containing 23 to 38 ng of DNA was approximately 10 ng and its recovery efficiency was ranged from 27 to 40% (Fig.2). Additionally, PCR using released DNA from microchip as a template was successfully achieved (Fig.3).

References

- [1] Cady, N.C., S. Stelick, and C.A. Batt, *Biosens Bioelectron*, **19**(1), 59-66 (2003)
- [2] Breadmore, M.C., et al. *Anal Chem*, **75**(8), 1880-6 (2003)
- [3] Yoza, B., M. Matsumoto, and T. Matsunaga, *J. Biotechnol*, **94**(3), 217-24 (2002).

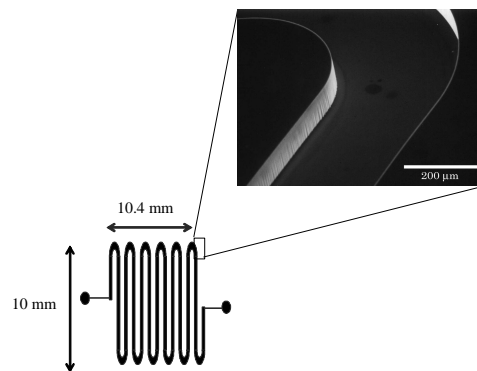


Fig.1 Layout of the microchip for DNA extraction. Channels are 300 μ m in width and 100 μ m in depth. Diameter of the inlet and outlet holes was 1 mm. Total surface area is 62 mm² and the bottom area is 37.2 mm².

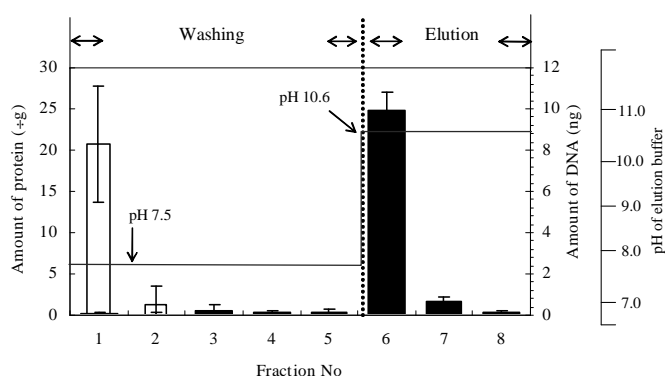


Fig.2 Profile of DNA and protein amount eluted from microchip during extraction from whole blood. Whole blood cells (5 μ l) were lysed in a solution containing 10 μ l of 10% Triton-X100 and 5 μ l proteinase K [10mg/ml]. Five μ l of the lysate of blood was injected. Non-specific binding and protein were washed with TE buffer; pH 7.5 (fraction 1-5) DNA was eluted with elution buffer; pH 10.6 (0.05 M sodium carbonate, 0.05 M sodium bicarbonate) (fraction 6-8). □: protein, ■: DNA

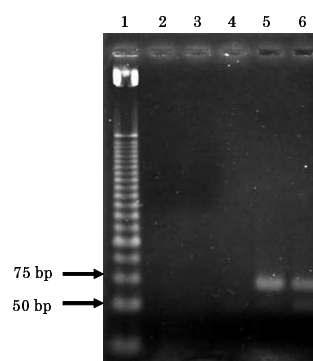


Fig.3 Agarose gel electrophoresis of PCR products (ALDH2 gene: 63 bp) amplified from whole blood DNA extracted by microchip coated with amine groups. Lane 1; 25 bp ladder, Lane 2; lysate of blood, Lane 3; non-trapped fraction, Lane 4; washing fraction; Lane 5; DNA eluted from microchip, Lane 6; DNA extracted by commercially available kit