Integrated Structure of PMMA Microchannels for DNA Separation Fabricated by Deep X-ray Lithography and Fusion Bonding

Yuichi Utsumi[a], Tomohiro Ikeda[b], Megumi Minamitani[b], Kazuo Suwa[b]
[a] Laboratory of Advanced Science and Technology for Industry, University of Hyogo
3-1-2, Koto, Kamigori, Ako-gun, Hyogo, 678-1205, JAPAN
[b] Fujipream Co., 38-1, Sikisai, Himeji, Hyogo, 671-2244, JAPAN
E-mail: utsumi@lasti.u-hyogo.ac.jp

Nowadays, the progress of life science have been increasing rapidly the importance of the micro fluidics for DNA analysis systems have been widely recognized especially in medical fields. Moreover it is very important to develop the high throughput DNA amplify technology and succeeding separation technology using electrophoresis to decode the genome in a short period for achieving the custom and order-made medical cares. The polymerase chain reaction (PCR) is an essential technique for the DNA assay of various disease and it has been strong requirement to shorten the whole PCR cycles more and more. The application of PCR reactor to microchip capillary electrophoresis (MCE) leads to the drastic shortage of the total time of analysis of DNA from very small quantity samples.

We developed the integrated structure of polymethylmethacrylate (PMMA) micro fluid channels consists of PCR well, DNA separation cahnnel, and heat exchanger channel using deeep x-ray lithography[1], molding and fusion bonding techniques by nanoimprint equipment. As for the design of the MCE, the separation resolution of DNA was intended to improve using the micro channel with variable width along with the corner of the channel. We simulated the diffusion of the sample plug during electrophoresis. As shown in Fig. 1 a) , a sample plug diffuses in accordance with the electrophoresis flow. This diffusion arose especcialy at the corner of the micro channel. In order to reduce the diffusion, micro channel with variable width along with the corner was designed. The diffusion of the sample plug was considerably suppressed as shown in Fig. 1 b) and c). The integrated structure of micro fluid channels consists of two micro channel chips. The desigin image is shown in Fig. 2 (a) and SEM image (b) of replicated structure of micro channels by nano-imprint technique. The diameter of the thermal cycler cell for PCR is 2.75 mm corresponding to the volume of 1 to 2 pl. Figuire 3 (a) shows the outside view of the stacked structure of PMMA micro fluid channels. The tacked structure contains 96 DNA analysis micro channels, and its size is 128 x 88mm square and 7 mm depth. Figure 4 shows the electropherograms for separation and detection of 100 DNA fragments by MCE-chemiluminescence. Capillary electrophresis was performed on a fluorescence microscopic apparatus equipped with a 488nm argon ion laser-induced fluorescence detection. Detection was carried out by measuring fluorescent at 534nm. A standard DNA stepladder marker (Bio-rad, USA) was used consisting 100 DNA fragments ranging from 100 to 1000bp in exactly 100-bp increments. The running buffer was 1/10 diluted solution of 10×TBE(0.89mol/l Tris-borate, 20mmol/l EDTA, pH8.3) from Wako and 0.01% GelStar Nucleic Acid Gel Stains (cambrex, USA) was used for a fluorescence detection of DNA. The length of the separation channel was 10.5mm and electrophoretic separation were performed at 0.75 kV. As shown in Figure 4, DNA fragments ranging from 100 to 1000bp was clearly separated using this integrated structure of micro fluid channels. An electrophoretic voltage was controlled with laboview.

We also evaluated the PCR property using PCR amplifification kit “TAKARA EX Tag”. The template was λ DNA and the length of the target DNA was 714 bp.

Reference: [1]Y. Utsumi, T. Kishimoto, J of Vac. Sci. and Technol. B, [23], 2903, (2005)

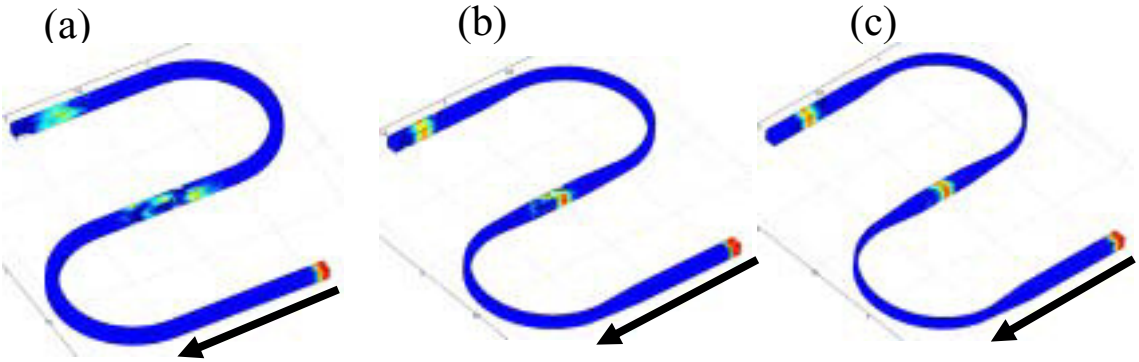

Fig. 1 Simulation resultc of the sample plug diffusion along with the electrophoresis flow. Depth of the channel was 80 μm.
(a) The width of the channel was constant value ; 80 μm.
(b) The width of the channel was reduced from 80 to 40μm at the corner.
(c) The width of the channel was reduced from 80 to 20μm at the corner.

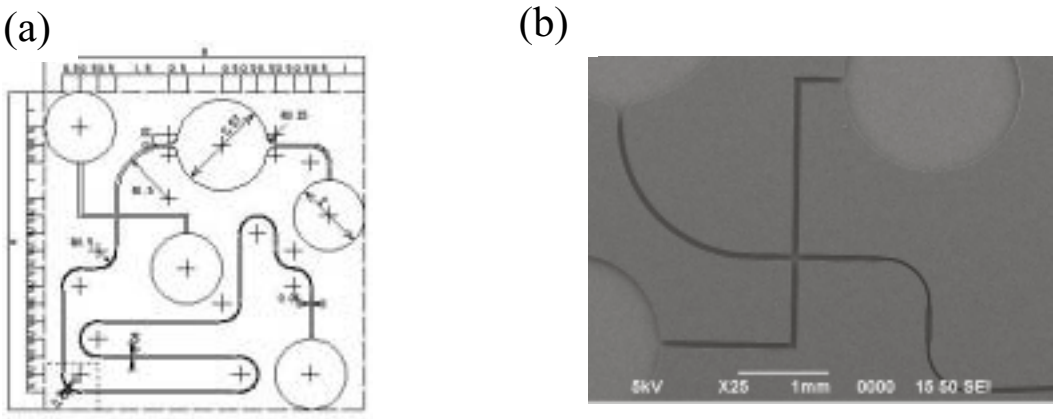

Fig. 2 (a) ; The desigin image and (b) : SEM image of replicated structure of micro channels by nano-imprint technique. The diameter of the thermal cycler cell for PCR is 2.75 mm, corresponding to the volume of 1 to 2 pl.

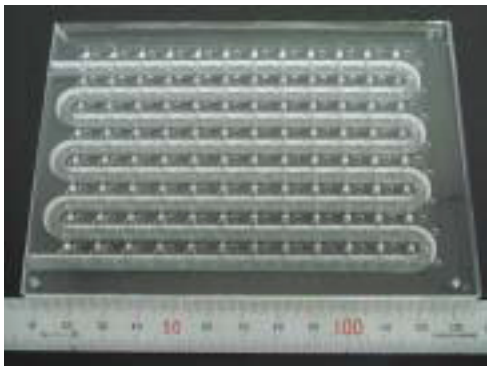

Fig. 3 Outside view of the stacked structure of PMMA micro fluid channels. The structure contains 96 MCE channels.

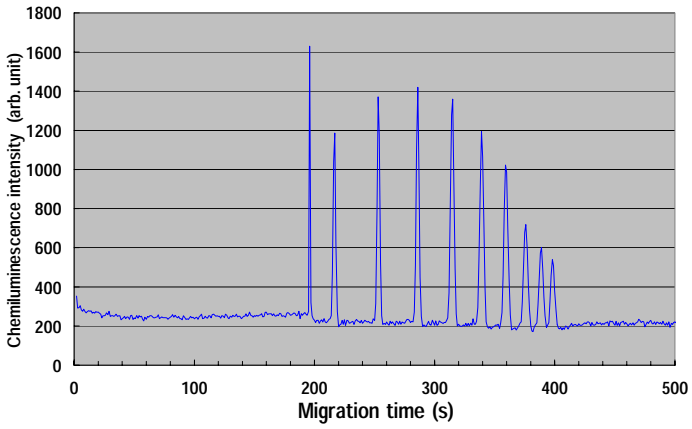

Fig. 4 Electropherograms for separation and detection of 100 DNA fragments by MCE chemiluminescence.