Fast DNA Analysis by Novel Separation Media based on Nanoparticles on a Microfabricated Chip

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Since human genome sequencing has been almost completed, human genome project will quickly move on to the post genome sequencing era, including single nucleotide polymorphism (SNP) analysis, functional genomics, mutation analysis, transcriptome analysis, proteome analysis, and metabolome analysis. Capillary array electrophoresis with 96-384 capillaries plays a vital role in the genome sequencing era, but in the post Human Genome sequencing era, further development of analytical technology for DNA, mRNA, protein, and is highly required. The microchip-based technology will be a key technology in the post-genome sequencing era [1-2]. But the research on the separation media suitable for microchip has still been remained [3-4], since the performance of the conventional separation media, such as polyacrylamide and methylecellulose, is unsatisfactory for ultra-fast analysis of DNA and protein.

A novel separation media, core-polymerized micelles (CPM) which is consist of methoxy-poly(ethylene glycol)/poly(lactide)-methacryloyl (MeO-PEG/PLA-MA), has been synthesized (Fig. 1). The size of nanospace, which is generated by the CPM of 53.3 nm diameter, can be easily controlled to adjust the CPM concentration and keep a low viscosity even if the concentration is very high. We had expected that these properties of the CPM solution made it possible to separate DNA more effectively in a microchannel.

After filling the CPM solution of 10 mg/ml in the microchannel (i-chip3, Hitachi Chemical), DNA sample solution was injected by using a novel pressurization technique, which could accomplish sample injection and concentration gradient of the CPM simultaneously in a separation channel (Fig. 2).

Resolution of DNA fragments, particularly over 5 kbp, was drastically improved in the CPM solution (Fig. 3A), whereas it was quite difficult to realize in polymer matrices, such as polyacrylamide, methylecellulose and so on. (Figs. 3B) The relationship between electrophoretic mobility and DNA size clearly illustrates the high-resolving power for larger DNA fragments in CPM solution.

To investigate electrophoretic behavior of DNA in the CPM solution, we performed direct observation of a migrating single DNA molecule [5] in the CPM solution and in gel by using fluorescence microscopy (Fig. 4). In the CPM solution, the DNA molecule migrates with maintaining a coiled conformation as in a free solution (Fig. 4A). It indicates that the mechanism of DNA separation in the CPM solution is fundamentally different from the traditional one, since the DNA molecule, larger than a few kbp, in agarose or polyacrylamide migrates with stretching and relaxing alternately as shown in Fig. 4B.

These results demonstrate the potential of a novel separation media itself and a confined nanospace gradient produced by pressurized technique for use in DNA separation, as well as protein, with high speed and high resolution by microchannel electrophoresis.

Figure 1: Schematic structure of methoxy-poly(ethyleneglycol)/poly(lactide)-methacryloyl (A) and AFM image (B) of a CPM.

Figure 2: Schematic drawing of the microchannel. Separation channel is filled with nanospace gradient. Channel dimensions are 100 μm wide and 30 μm deep.

Figure 3: Electropherograms of 1 kbp ladder (1-15 kbp) in 10 mg/ml CPM solution (A) and a long 5 kbp ladder (0.5–5 kbp) in 1% methylcellulose solution (B).

Figure 4: Sequential fluorescence images of T4 DNA (165.6 kbp), which are migrating in 10 mg/ml CPM solution (A) and 1% agarose gel (B).