High-Speed Analyzing PCR Products of *M. tuberculosis* Genome Stained by Ethidium Bromide on Microchip Gel Electrophoresis

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The technique of microchip gel electrophoresis (MCGE) was used to analyze the polymerase chain reaction (PCR) products of *M. tuberculosis* Genome stained by ethidium bromide. The electrophoretic process was completed within 3—4 min and the results show that the technique of microchip electrophoresis is a high-speed and high-sensitivity analyzing method.

Keywords high-speed, *M. tuberculosis*, microchip gel electrophoresis

Introduction

Generally the methods of agarose gel slab electrophoresis (AGSE) and polyacrylamide gel electrophoresis (PAGE) are used in the analyses of the request of analysis PCR products and other DNA fragments. But resolution and separation efficiency of the AGSE are low and the detection is less sensitive. So it is difficult to analyze the PCR products, which have multiple fragments with the length difference within only several base pairs, and the samples, which concentration is only pg/μL, can not be analyzed. PAGE can match this request, but the process of electrophoresis and silver staining is very complex and the analysis is time consumption.[1] Certainly, capillary electrophoresis is an effective technique to analyze DNA fragments. But, the analyzing time is about half an hour or longer. So a high-speed and high-sensitivity analysis method is desirable.

In recent several years, microfabricated capillary electrophoresis chip has been introduced.[2,3] Now it has been used for rapid analysis of biologically relevant samples, such as DNA restriction fragments,[4,5] PCR products,[4] DNA sequencing fragments,[6] and short oligonucleotides.[7] In this paper, it was reported that the technique of microchip gel electrophoresis (MCGE) was used to implement high-speed and high-sensitivity DNA fragments analysis.

Experimental

Materials

The fused-silica microchip was microfabricated using standard photolithography, wet chemical etching and bonding techniques.[8] The schematics of the microchip is shown in Fig. 1. The channel on the microchip is 30 μm deep and 90 μm wide at the top, the effective separation length is 8 cm and the three side channels have a length of 5 mm. The volume of the cross-T injector is 1.17×10^{-4} μL. The channel on the microchip is coated with linear polyacrylamide (LPA) using a modified Hjerten procedure.[9]

The pGEM-7Zf(+)DNA/HaeⅢ Markers and pBR322 DNA/HaeⅢ Markers were purchased from Sino-American Biotechnology Company. The *M. tuberculosis* Genome was supported by the Disease Control Center of Shanghai. Other reagents used in this experiment were acrylamide, ethidium bromide, *N*,*N*,*N*′,*N*′-tetranethylethylenediamine (TEMED), ammonium persul-

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fate (Shanghai Sangon Company), *AmpliTaq* DNA polymerase (Perkin Elmer, Branchburg, New Jersey) and [γ-(methacryloxy)propyl]trimethoxysilane (Sigma, St, Louis, MO).

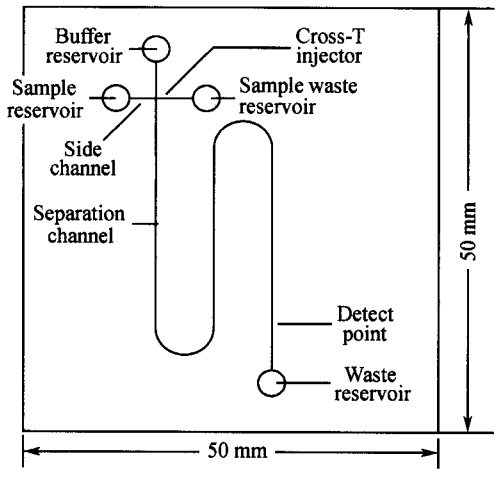

Fig. 1 Schematic of the fused-silica microchip (it has a simple cross-T injector and an 8-cm separation length, the reservoirs are configured for sample pinched injection).

Instrument

Channel performance and separation were monitored on-microchip using a single point detection scheme via a laser confocal fluorescence detection system.[10] An argon ion laser (488 nm, Coherent Innova 90) was used for excitation and focused within the channel using a 20 × NA0.5 microscope objective. Fluorescence was collected by the objective lens and passed through band-pass filter (570—620 nm), then was focused through 500-μm confocal pinholes with a 100-mm focal length lens and measured using a photomultiplier tube (PMT, Oriel 77340). The signal from the PMT was collected and processed by a set of filter and amplifier circuit board, then inputted to the computer by a 12-bit ADC board and displayed the spectral on the screen by a program written by ourselves. Platinum electrodes provided electrical contact from power supply to the solutions in the reservoirs.

Sample preparation and microchip gel electrophoresis

Four electrophoretic DNA samples were prepared: (1) pGEM-7Zf(+)DNA/HaeⅢ Markers in pure water with the final concentration of 2 ng/μL; (2) pBR322 DNA/HaeⅢ Markers having the same concentration of 2 ng/μL; (3) PCR products of *M. tuberculosis* Genome; (4) a mixture of 15 μL of sample (1) and 15 μL of sample (3). Before electrophoresis, each of DNA samples (30 μL) was stained by adding with 1% (*V*/*V*) ethidium bromide (2 μL) and incubating for 15 min in the dark. The *M. tuberculosis* Genome, as the template, was amplified in a 50 μL of reaction mixture containing 10 × buffer, 4 × dNTP's, four pairs special primers, 1.5 units of *AmpliTaq* DNA polymerase and pure water. PCR amplification followed the protocol: 94 ℃ denatured for 4 min, and 30 cycles of 94 ℃ for 40 s, 55 ℃ for 1 min, and 72 ℃ for 40 s, final 72 ℃ extension for 5 min.

The sieving matrix was 4% or 3% linear polyacrylamide polymerized in-house using 0.01% (*W*/*V*) ammonium persulfate as initiator and 0.01% (*V*/*V*) *N*,*N*,*N*′,*N*′-tetranethylethylenediamine (TEMED) as catalyst. It was prepared in a buffer of Tris-boric (40 mmol/L) acid EDTA (1 mmol/L), pH 8.0 (referred to as 1 × TBE).

The sieving matrix was full-filled into the microchannel and pre-electrophoresis for 20 min. Then loadded 5 μL of the DNA samples, which had been stained by ethidium bromide, into the sample reservoir. The electrophoretic conditions of all the DNA samples were the same. With electrokinetic introduction the sample was introduced into the cross-T injector. The injecting electric field was 200 V/cm and injection time was 30 s. Then the sample was separated by the microchip gel electrophoresis with the separation electric field that was 200 V/cm. The electrophoresis signal was detected online as illustrated above.

Results and discussion

Fig. 2 presented the microchip electrophoregram of the pBR322 DNA/HaeⅢ Markers. It demonstrated the excellent resolution was obtained with the techniques. The fragments of the Markers were well separated. As the injection mode introduced above, the injection volume was about the volume of the cross-T injector, which was 1.17×10^{-4} μL. From the electrophoretogram, as for the peak-15 (267-bp), the concentration was 25.3 pg/μL, the limit of detection was up to 1.65×10^{-20} mol (S/N = 2). Also from the electrophoretogram it was found that only the smallest 8-bp DNA fragment concentration was not high enough for successful detection and the 123-bp/

124-bp fragments migrated together. All the DNA fragments had being detected within 4 min. The 184-bp, 192-bp, 213-bp, 234-bp and 267-bp fragments had been up to baseline resolution. The resolutions between the peaks of 11, 12, 13, 14 and 15 were 3.12, 4.16, 4.21 and 6.13, respectively. All of these demonstrated that the separation speed, limit of detection and resolution of the microchip system were much higher than agarose gel slab electrophoresis, PAGE, especially for the short fragments. Compared with capillary electrophoresis (CE), the microchip electrophoresis was easy to control the injection volume, the separation length was shorter, and the analyzing time was shorter. But the resolution was not lose, it was more than 3. So the analysis speed of microchip gel electrophoresis was higher than that of CE.

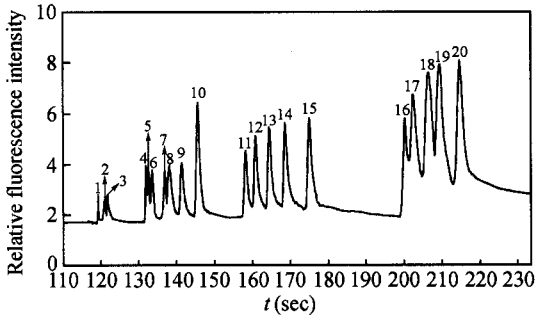

Fig. 2 Electrophoretogram of the pBR322 DNA/HaeⅢ Markers using microchip gel electrophoresis [sieving matrix: 4% linear polyacrylamide prepared in 1 × TBE buffer, separation electric firld: 200 V/cm, peak identification: (1) 11-bp, (2) 18-bp, (3) 21-bp, (4) 51-bp, (5) 57-bp, (6) 64-bp, (7) 80-bp, (8) 89-bp, (9) 109-bp, (10) 123/124-bp, (11) 184-bp, (12) 192-bp, (13) 213-bp, (14) 234-bp, (15) 267-bp, (16) 434-bp, (17) 458-bp, (18) 504-bp, (19) 540-bp and (20) 587-bp].

According to the purpose of the experiment, four pairs of special primers matching the template *M. tuberculosis* were designed, and added into the PCR reaction mixture. Four amplified DNA fragments should be obtained. They were katG1 (190-bp), katG2 (467-bp), katG3 (390-bp) and katG4 (321-bp), respectively. When loaded the amplified products and electrophoresis on the agarose gel, it was observed that there was almost no signal under UV-light because the concentration was too low. But after detecting the amplified products on the microchip system, the result shown in Fig. 3 was got. In Fig. 3, there were three electrophoretograms of the pGEM-7Zf(+)DNA/HaeⅢ Markers, PCR amplified product and the mixture of the two samples, respectively.

Fig. 3A showed the standard size of the Markers. From Fig. 3B, it was observed that there were two peaks by use of microchip gel electrophoresis. Comparing to the standard size of the pGEM-7Zf(+)DNA/HaeⅢ Markers and the migration time of the peaks, it was deduced that peak (a) was 190-bp and peak (b) was 467-bp, respectively. And katG1 and katG2 had been well amplified, but katG3 and katG4 had not well amplified or amplified inefficiently. Fig. 3C gave the more evidence. It was the electrophoretogram of the mixture of the Markers and PCR products. Therefore this method could be used to detect the efficiency of PCR amplify.

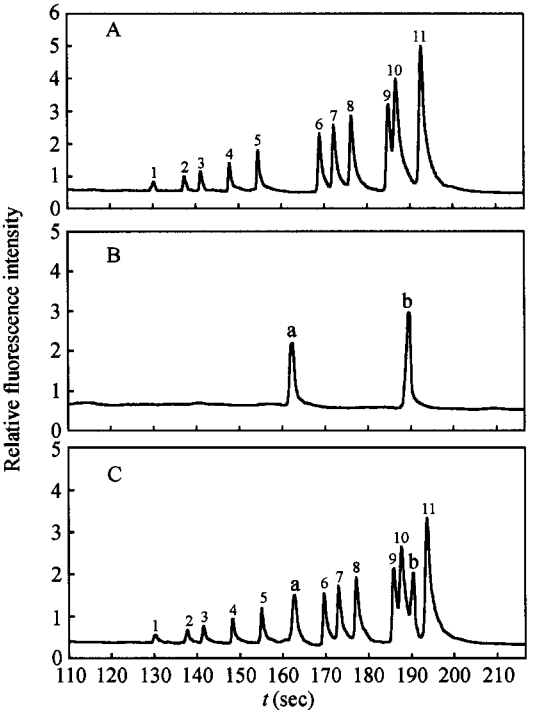

Fig. 3 Electrophoretograms using microchip gel electrophoresis [sieving matrix: 200 V/cm; (A) pGEM-7Zf(+) DNA/HaeⅢ Markers, peak identification: (1) 40-bp, (2) 80-bp, (3) 102-bp, (4) 142-bp, (5) 174-bp, (6) 267-bp, (7) 289-bp, (8) 328-bp, (9) 434-bp, (10) 458-bp and (11) 657-bp; (B) PCR products, peak identification: (a) 190-bp and (b) 467-bp; (C) mixture of pGEM-7Zf(+)DNA/HaeⅢ Markers and PCR products, peak identification: the same as (A) and (B)].

Conclusions

In this paper, a method of high-speed and high-sensitivity DNA fragments analysis on the microchip gel electrophoresis was reported. Using this method, the pBR322 DNA/HaeⅢ Markers, pGEM-7Zf(+)DNA/HaeⅢ Markers and the PCR products of *M. tuberculosis* Genome had been analyzed. The result demonstrated that the excellent resolution was obtained, and the whole electrophoretic process was completed within 4 min. The limit of detection was up to 1.65×10^{-20} mol (S/N = 2). The separation speed, limit of detection and resolution of the MCGE were better than those of AGSE and PAGE. Compared with CE, the microchip electrophoresis was easy to control the injection volume, the effective separation length was shorter, and the analyzing time was more short. From the electrophoretic results of PCR products of *M. tuberculosis* Genome, it could be obtained that katG1 and katG2 had been well amplified and katG3 and katG4 amplified inefficiently.

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