Poly(dimethylsiloxane) based microchip for DNA electrophoresis*

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Abstract A novel poly(dimethylsiloxane)(PDMS)-based microchip for DNA separation through electrophoresis has been developed using a micro-electro-mechanical-system(MEMS) technology. Unlike previous hybrid PDMS microchip, one PDMS film is first created on glass support by pressing method in our microchip. Thus, increased band-broadening phenomena arising from the material nonuniformity at the walls of microchannel can be avoided in electrophoresis process. A low-viscosity hydroxypropylmethylcellulose-100 (HPMC-100) is used as the separation medium for fluorescent intercalator-labeled double-stranded DNA (dsDNA) fragments. Mannitol is introduced to PDMS-based microchip as a separation medium additive to enhance separation efficiency. At applied electric field strength of 150 V/cm, excellent separations of the PCR marker could be achieved with an effective separation distance of 25 mm.

Keywords: poly(dimethylsiloxane)(PDMS), micro-electro-mechanical-system (MEMS), DNA fragments, electrophoresis microchip.

Micro-electro-mechanical-system (MEMS) technology had recently entered the forefront of analytical chemistry and life science[1]. Due to its advantages of high throughput and high efficiency, it minimized reagent consumption and reduced cost[2]. Microchip-based electrophoresis technique was one of the most important steps in the development of bioMEMS. Most electrophoresis microchips had been fabricated using well-established semiconductor technology from silicon or glass substrates[3,4]. However, the fabrication process was expensive, time consuming and labor intensive. Alternatively, polymer substrates, including poly (dimethylsiloxane) (PDMS) and polymethylmethacrylate (PMMA), were promising materials for building microchips. Since Effenhauser et al.[5] first reported PDMS electrophoresis microchip in 1997, PDMS have been widely accepted as an excellent candidate material for microchip applications. Generally, complete PDMS microchips, composed of PDMS replica and PDMS wafer, are very pliable and special care should be taken in experiment. The rigid characteristics can be enhanced in hybrid PDMS microchips made from PDMS replica and another flat substrate material, such as glass[6], PMMA[7]. However, due to the nonuniformity of surface charge density at the walls of the channels, increased dispersion has been introduced in hybrid microchips[7,8].

In this work, we develop a pressing method to form a PDMS thin film on glass support and construct a novel PDMS-based microchip. As there is one PDMS thin film in our microchip, increased band-broadening phenomena from the material nonuniformity do not occur and separation efficiency may be improved. In DNA separation through electrophoresis, mannitol is introduced to PDMS-based microchip as a separation medium additive. The satisfactory resolution has been achieved in PDMS-based microchip by using 1.8% HPMC-100 with 6% mannitol added.

1 Experimental

1.1 Materials and reagents

PDMS was purchased from Dow Corning (Midland, MI). Tris (hydroxymethyl) aminomethane (Tris), boric acid, and ethylenediaminetetraacetic acid (EDTA) were obtained from Fluka (Buchs, Switzerland). Hydroxypropylmethylcellulose-100 (HPMC-100, the viscosity of which is 100 cp in 2% aqueous solution) and mannitol were received from Sigma (St. Louis, MO, USA). PCR marker was purchased from the Sino-American Biotechnology Company (Beijing, China). The PCR marker contained 6 double-stranded fragments with 237, 377,

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515, 697, 994, and 1543 bp. DNA sample was diluted with deionized water to a concentration of about 50 μg/mL and stored at −20 ℃. SYBR Green I (concentration not given) in dimethyl sulfoxide was purchased from Molecular Probes (Leiden, The Netherlands). 1 × TBE buffer, composed of 89 mmol/L Tris, 89 mmol/L boric acid and 2 mmol/L EDTA, was prepared in laboratory. HPMC-100 and mannitol were dissolved in 1 × TBE buffer and stored overnight at 4 ℃ until the solution appeared homogeneous and transparent. Prior to use, the sieving matrix was filtered through sterile 0.45 μm pore size filters and degassed. Unless otherwise indicated, all solutions were prepared using deionized water in experiment.

1.2 Instrumentation

Signals were attained by a laser induced fluorescence (LIF) detection technology. The detection system was constructed through modifications of a commercial inverted microscope (BX-51, OLYMPUS, Japan) with a filter cube for fluorescence observation, an 1× 10 objective (UPlanFI, OLYMPUS, Japan), and a cooled CCD camera (DP50, OLYMPUS, Japan)[9]. A 100-W mercury lamp (USH102D, USHIO Inc.) was used as excitation light source. The fluorescent emission was registered by a CCD camera. The PDMS microchip assembly was mounted on the *X-Y* translational stage of a reflected microscope, which also served as a platform of a laser-induced fluorescence detection setup. Two high electric power supplies (ECP3000, Liaya Instrument Factory, Beijing, China), which were connected to four platinum electrodes, were used to provide high voltages in this work.

1.3 PDMS-based microchip fabrication

The method used to build PDMS-based microchip, apart from the creation of PDMS film on glass substrate, was similar to previously reported procedures[10, 11]. The overview of the process for fabrication of PDMS-based microchip could be seen in Fig. 1. Briefly, a plain glass wafer was spin-coated with a negative photoresist SU-8. The spinning speed determined the thickness of the SU-8 coating and thus the channel depth. A negative chrome mask, which contained the desired channel features, was utilized in photolithographic process. The unexposed SU-8 was flushed off with a SU-8 developer, leaving the SU-8 structures standing on the glass wafer. In order to ensure PDMS wafer peels off easily from SU-8 master, the master was first silanized by placing it in desiccators under vacuum for 15 minutes along with a vial containing a few drops of silanizing agent. A 10 :1 mixture of PDMS oligomer and cross-linking agent (Sylgard 184), which had been degassed under vacuum, were poured onto the master. After at least 1 hour of curing at 80 ℃, PDMS replica with microchannel was removed from the master and reservoirs were cut out with a hole punch.

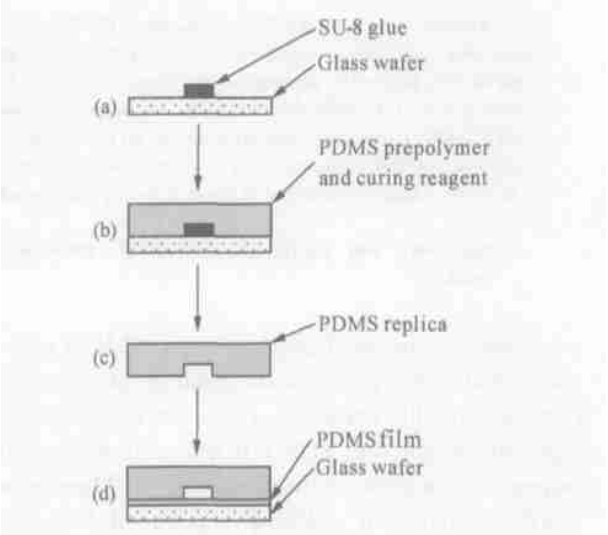

Fig. 1. Fabrication procedure of PDMS-based microchip for capillary electrophoresis. (a) Master wafer with a positive relief of SU-8 photoresist, (b) a prepolymer of PDMS and its curing agent was then cast over the master wafer and cured at 80 ℃ for 1 h, (c) PDMS reptica was peeled from the master wafer; and (d) PDMS replica and a PDMS film on glass support were put together in conformal contact to seal reversibly.

To form a PDMS thin film on the glass wafer, two pieces of thin polymer film with 30 μm thickness were first placed on two ends of the glass wafer as supports. Then several drops of PDMS prepolymer were poured over one glass wafer which was not silanized and another silanized glass wafer was used as a cover to press PDMS prepolymer solution. Afterward, a pressure was applied to between two glasses in our in-house equipment and excessive PDMS prepolymer was extruded, leaving one PDMS film. After the remaining PDMS film was cured in an oven at 80 ℃ for 1 h, the silanized glass was easily peeled off and PDMS film with about 30 μm thickness was hermetically stuck to the unsilanized glass. The thickness of PDMS film was determined using Alpha-step® 500 Surface profiler in our laboratory. Then, PDMS replica and glass support with PDMS film were put together in conformal contact to seal reversibly.

Schematic diagram of PDMS-based microchip is shown in Fig. 2. It contained a 35 mm long separation channel and a 10 mm long sample injection channel. The channels are 100 μm wide and 20 μm deep. The injection channel crosses the separation channel connecting reservoirs 2 and 4 in a 200 μm-long double-T arrangement.

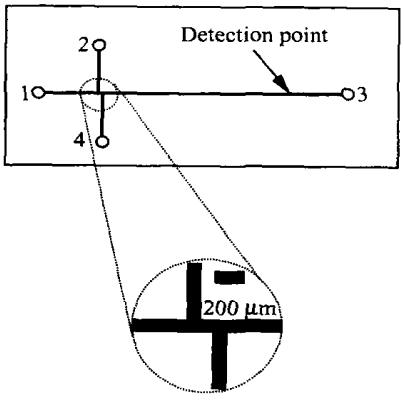

Fig. 2. Schematic diagram of PDMS-based microchip. The distances from the crossing point to reservoir 1 (buffer), reservoir 2 (sample), reservoir 4 (sample waste), reservoir 3 (waste) and detection point were 5, 5, 5, 30 and 25 mm, respectively. The channels were 100 μm wide and 20 μm deep. The injection channel crossed the separation channel that connected reservoirs 2 and 4 in a 200 μm-long double-T arrangement and a detailed description of it is shown in the inset.

1.4 Microchip electrophoresis procedures

The separation medium consisted of 1 × TBE buffer, 1.8% w/v HPMC-100 and 6% w/v mannitol. For visualization of microchannel, the intercalating dye SYBR Green Ⅰ was added to the separation medium at a dilution 1∶10000. In the sample loading procedure, 180 V was applied to the sample waste reservoir 4 and the sample reservoir 2 grounded while other reservoirs were floated. Following a 70 s sample injection, subsequent separation occurred by applying a potential of 525 V between reservoirs 1 and 3. This results in separation field strength of 150 V/cm. DNA separation imaging was recorded at detection point with 5 mm far from reservoir 3, corresponding to 25 mm effective separation distance.

2 Results and discussion

As an excellent candidate material for microchip electrophoresis applications, PDMS showed almost ideal characteristics[12, 13]. In addition, it had some addition advantages over other substrate materials. For example, electroosmotic flow in PDMS-based microchip was dramatically decreased compared to glass microchip; therefore, there was no need for surface modification which was inherent with glass microchip to eliminate the influence of electroosmotic flow[14]. PDMS could make reversible van der Waals contact to a variety of materials, including glass, silicon and PMMA[15]. Thus, once PDMS microchip was clogged, it could be easily peeled off from the support wafer, rinsed with deionized water and sealed again after drying at room temperature.

Generally, there were two types of formats in PDMS-based microchips shown in Fig. 3. One was complete PDMS microchip, which was composed of PDMS replica and PDMS wafer. Since PDMS material was very pliable compared to other microchip materials, an additional support wafer was required to facilitate experiment operation. The other was hybrid PDMS microchip, in which PDMS replica sealed with different materials, such as glass, silicon and PMMA. As glass material had a five-fold greater heat conductivity k compared to PDMS, the capability of hybrid PDMS microchip to dissipate heat was much better than that of complete PDMS microchip. However, materials nonuniformity at the walls of the channels was introduced in hybrid microchip. It is well known that electroosmotic phenomenon results from the formation of an electric double layer at the microchannel walls. The electric double layers arise when certain groups, such as carboxylate group, silanol group, on the surface of microchannel substrates were ionized in the presence of the buffer. Since PDMS and glass had completely different surface charge density, the separation effectiveness of hybrid microchips had been questioned[7, 8].

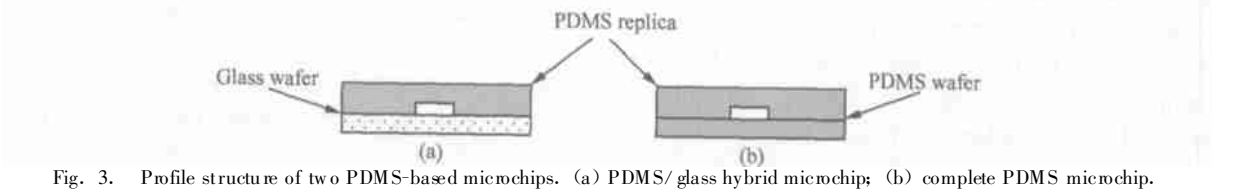

Fig. 3. Profile structure of two PDMS-based microchips. (a) PDMS/glass hybrid microchip; (b) complete PDMS microchip.

To further address this issue, creating a PDMS film on glass support was first considered, as PDMS thin film had better ability to dissipate heat than thick PDMS wafer. However, the experiments showed that it was very difficult to form flat PDMS thin film on glass wafer using spin coating method. There were two major reasons to this deficiency. First, PDMS pre-polymer was one kind of viscous liquid and handling it became very troublesome. Previous study showed that even when spinning wafers at 8000 rpm, the thickness of PDMS thin film was greater than 40 μm[16]. Secondly, even if the PDMS film could be formed on the glass wafer by spin coating method, it was also impossible to gain a smooth PDMS surface in that there were strong surface tension and bulk shrinkage from PDMS pre-polymer when it was gradually solidified. Thus, sealing failure might occur due to uneven PDMS surface. In this work, a pressing method was first developed and adopted to form PDMS film on glass wafer. To effectively remove glass wafer without destroying PDMS film, one of the two glass wafers was first silanized and the other was not. Thus, when PDMS pre-polymer was polymerized, the cured wafer was removed with ease and PDMS film was hermetically stuck on the uncured glass wafer.

Hydroxypropylcellulose (HPC) had been used as separation medium for DNA separation in native or oxided PDMS-based microchips[5,17]. Some investigators had also examined what the polyols in the TBE buffer markedly improved the separation efficiency of DNA fragments using HPMC as sieving media[18,19]. They attributed the effects to a change in the sieving pore size in the separation medium by the complexation of borate with polyols (glycerol or mannitol) and cellulose derivatives (such as HPMC), in which the borate ion acted as a bridge between polyol and HPMC. In this paper, HPMC was used as separation medium and mannitol was introduced to PDMS-based microchip as an electrophoresis separation medium additive to enhance separation ability. We observed that the migration time dramatically increased and separation efficiency significantly enhanced along with mannitol added. This observation was consistent with previous results by Ren J. supporting that addition of mannitol increased the viscosity of the buffer and decreases pH of the buffer[20]. Fig. 4 shows a separation image of the 1543-bp, 994-bp and 697-bp fragments in PDMS/glass hybrid microchip and our novel microchip. As could be seen, DNA fragments could be completely resolved under above-mentioned electrophoresis conditions in both microchips. However, by comparison of the separation imaging of two microchips, we found that increased band-broadening phenomenon had not occurred in novel microchip and it was attributed to the formation of PDMS film on the glass wafer in this microchip. It should also be pointed out that the other DNA fragments, which were also well-separated, were out of sight of the objective as 10× objective was used in our LIF detection system.

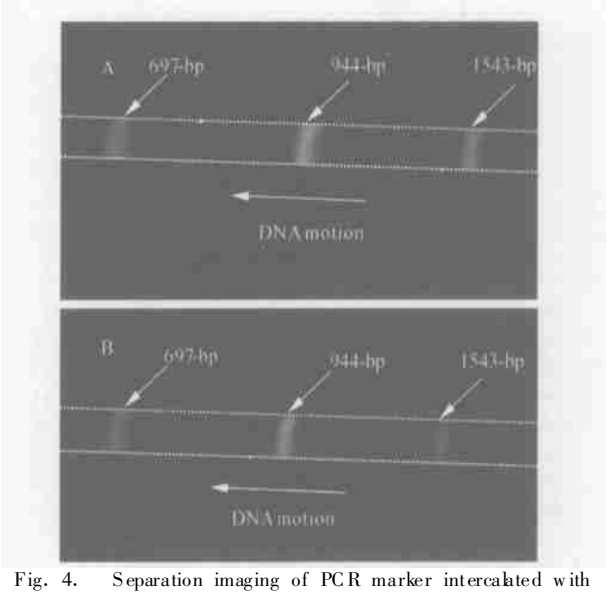

Fig. 4. Separation imaging of PCR marker intercalated with SYBR Green I in PDMS-based microchips. The separation medium contained 1× TBE buffer, 1.8% HPMC-100 and 6% mannitol and SYBR Green I was added to separation medium for labeling DNA fragments at a dilution 1∶10000; the separation electric field strength was 150 V/cm and DNA concentration was 50 μg/mL. (a) In hybrid PDMS microchip; (b) in our novel PDMS microchip.

3 Conclusions

A novel PDMS-based microchip was constructed using well-established MEMS technology in the present study. Due to the formation of a PDMS film on glass support in microchip, increased band-broadening phenomena, which occurred in hybrid PDMS microchip, were avoided. As a demonstration of microchip applications, DNA electrophoresis separations were achieved by using 1.8% HPMC with 6% mannitol added. For PDMS microchip electrophoresis, no column coating was needed which would otherwise require tedious derivatization procedures. Besides, mass production of disposable microchips was possible due to the low cost PDMS material, easy microfabrication process and reliable replication techniques.

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