SINGLE-STEP CAPILLARY ELECTROPHORESIS FOR FIELD-AMPLIFIED SAMPLE STACKING

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ABSTRACT

We developed a novel single-step capillary electrophoresis (SSCE) scheme for field-amplified sample stacking (FASS) by using a hydrophilic material polymethyl methacrylate (PMMA) microchannel chip. The capillary effect was used to introduce liquids and control capillary stop valves in a partial barrier structure of the microchannel. Through the combined action of stop valves and air vents, both sample plug formation for electrophoresis and sample injection into a separation channel were successfully performed in a single step. To optimize SSCE, different stop valve structures were evaluated. Using this method, DNA ladder was concentrated 40 fold and separated well within 1 min.

KEYWORDS

capillary effect, capillary stop valve, field-amplified sample stacking, microchip capillary electrophoresis

INTRODUCTION

A electrophoretic separation can be used for diagnostic applications such as multiplex molecular diagnostics, PCR product detection [1], and immunoassays, which involves detection of electrophoretic mobility shifts between bound and unbound proteins [2]. Since a short turnaround time and automated operation are important in the field of diagnostics, microchip capillary electrophoresis should be improved to further reduce analysis time, operation steps, and equipment size, and signal enhancement as well. Several studies have demonstrated sample concentration in a microchannel [3]. However, these are multistep processes that require complex coating or packing of porous polymers into the microchannel.

We developed a novel SSCE scheme for FASS by using an injection molded microchannel chip, which was made from the hydrophilic material PMMA, equipped with a capillary stop valve. Taking the surface tension property of liquids into consideration, the capillary effect was used to introduce liquids and control capillary stop valves in a partial barrier structure in the wall of the microchannel. Through the combined action of stop valves and air vents, both sample plug formation for electrophoresis and sample injection into a separation channel can be performed in a single step (figure 1). The FASS scheme is based on the simple principle that ions in a high electric field migrate faster than those in a low electric filed. In the FASS scheme, a low-concentration buffer is used as the sample solution, and thus, the sample ions can be concentrated at the boundary of 2 buffers (figure 2).

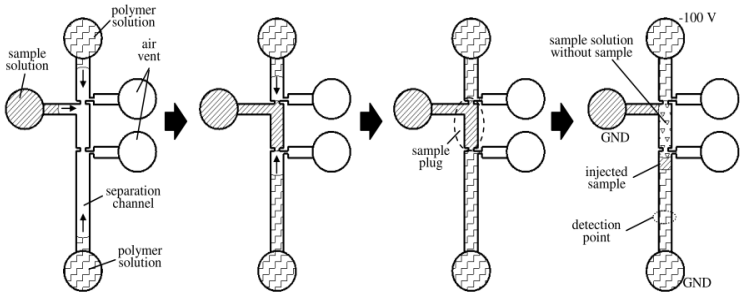

Figure 1. Principle of an SSCE scheme with capillary action and stop valve. The area indicated by diagonal lines and zigzag lines indicate 2 different liquids. The voltage indicated in this figure is a representative value. Different voltages are applied in actual use.

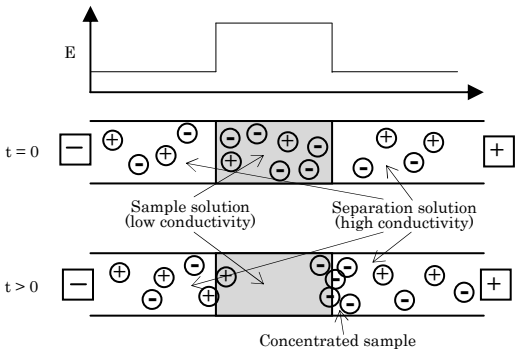

Figure 2. Principle of FASS scheme

EXPERIMENT

All PMMA microchannel chips used in this research were kindly supplied by the Enplas Corporation (Tokyo, Japan). Injection-molded PMMA microchannel chips were thermally bonded to 125-μm–thick PMMA film by Enplas. The stop-valve function was evaluated with varying types and sizes of partial barrier valve structures. The partial barrier structures were located on the channel sidewall and on the bottom of the channel. As shown in the scanning electron microscopy images (Fig. 3), round and square partial barriers were present in the side-wall. Partial barrier sizes varied from 5–35 μm for the round type and 15–45 μm for the square type, and bottom partial barriers with sizes ranging from 0–9 μm were fabricated and their stop-valve functions were evaluated. The main channel width was 100 μm with a depth of 30 μm. The PBS solutions having different tween-20 concentration PBS buffers were introduced into the microchannel using the capillary effect, and whether the meniscus stopped at the valve within 10 min after liquid introduction was observed. Furthermore, shock tests were performed to evaluate valve

stability. In the shock test, the chips were dropped onto the floor from a height of 1 m and the meniscus was observed subsequently.

Evaluation of SSCE was performed using a 20-µm round-type stop valve with a side partial barrier and a 3-µm bottom partial barrier to stabilize valve performance and to minimize sample plug deformation. All other channel widths and depths were 100 µm and 30 µm, respectively. Channel length for the sample plug was 3 mm and the length of the separation channel was 12 mm. The detection point for the electropherogram was 1.8 mm from the sample plug channel. DNA ladder separation with SSCE was observed using a conventional fluorescent microscope (BX51; Olympus, Japan) and CCD camera (DXC-390; Sony, Japan). Video was captured using a video capture device (K-BD; KEIAN, Japan); sample brightness was evaluated using in-house software. A separation solution for DNA was prepared by dissolving 2% (w/v) HEC (SP600, DAICEL Finechem LTD., London, England) into TBE solution. The concentration of the DNA ladder for SSCE evaluation was adjusted to 5 µg/mL by dissolving 0.05× TBE sample solution with 0.5% HEC and 1× SYBR Green I (Life Technologies Corporation, Carlsbad, CA, USA). TBE buffers were prepared by diluting 10× TBE with deionized water at several different ratios to evaluate various FASS buffer systems.

RESULTS AND DISCUSSIONS

Figures 3a and 3e show the success rate of stop-valve functions with different types and sizes of partial barriers without Tween-20 PBS solution. Success rates were obtained from 10 experiments. For the round-type partial barrier valve, most valves did not function properly in the absence of the bottom partial barrier structure. However, valve function Shock tests were performed to evaluate valve stability (Fig. 3b and 3f). Shock effects occurred primarily for the 3-µm small bottom partial barriers, with minimal effects observed with bottom partial barriers bigger than 6 µm (Fig. 3b). The valve with a 20-µm side partial barrier with a 3-µm bottom partial barrier is the smallest valve that showed a stable valve function and a minimum difference in the channel cross section. In contrast, square-type side partial barriers worked well without the bottom partial barrier in PBS solution. Even after the shock test, the valve functioned stably with the square-type partial barrier when the bottom partial barrier was fabricated (Fig. 3f).

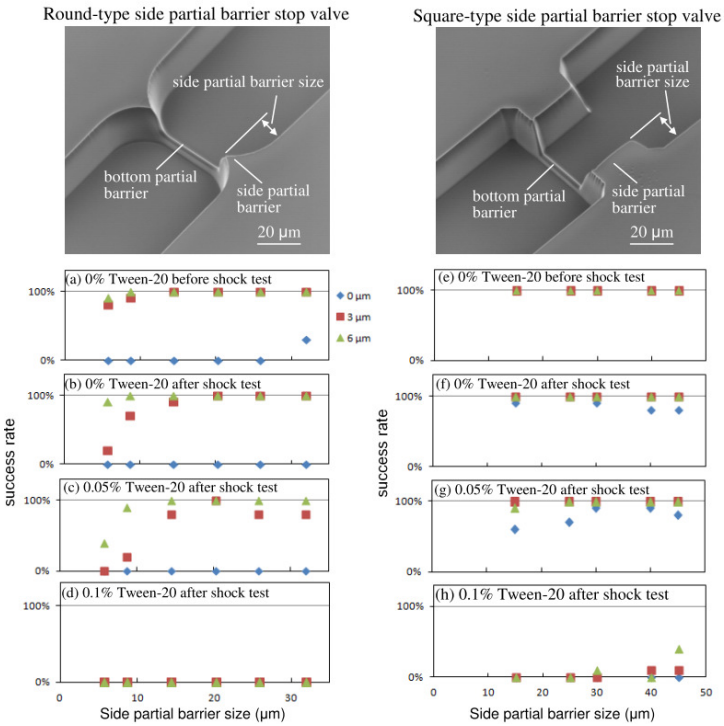

Figure 3. Comparison of capillary stop valve success rate using different types and sizes of side and bottom partial barrier structure.

The same tests were performed in PBS solutions containing 0.05% and 0.1% Tween-20. Only the shock test results are indicated for PBS containing Tween-20 (Fig. 3c). The success rate decreased as Tween-20 concentration increased and any partial barrier structure was able to stop the liquid at a Tween-20 concentration of 0.1%. Since partial barrier structures were located only on the side and the bottom of the channel in this study because the design was simple and cost-effective, the top of the channel was unable to stop the liquid. To use a liquid with a contact angle of less than 50 degrees, a partial barrier on the top of the channel is necessary.

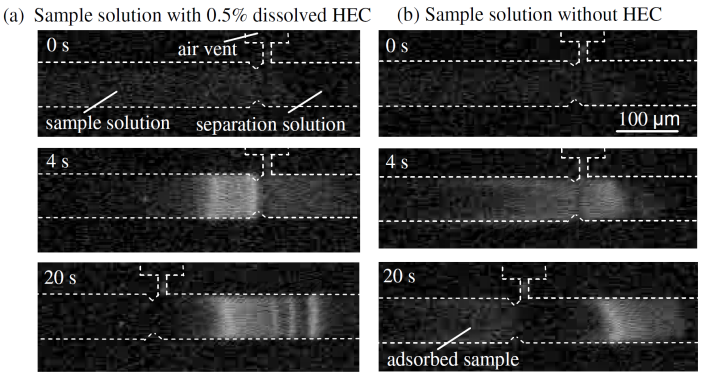

Figure 4. Images of SSCE operation with sample solution containing 0.5% dissolved HEC (a) and without HEC (b). Dotted lines indicate the channel wall.

Images of the SSCE operation are shown in Fig. 4. All liquids were introduced by capillary action within 2 min, and the liquids stopped at the liquid stop channels. Next, sample plug formation and injection into the separation channel were successfully performed using single-step electrical operation. By adapting the bottom partial barrier, the side partial barrier size can be minimized and a flat form sample plug can be introduced into the separation channel. Since the sample plug was concentrated in a flat form, separation was observed by 20 s for a short channel length.

Figure 4b shows the results obtained using a sample solution that did not contain 0.5% HEC. In this case, sample plug deformation and sample adsorption were observed. A native polymer surface, such as PMMA, PC, PDMS, or COP, has zeta potential despite the absence of ionic function the polymer surface. By applying a voltage to the microchannel, the zeta potential generates a flow in the microchannel, or EOF. This flow can be dramatically reduced by using a small amount of additive such as HPMC and HEC by self-coating [4]. When a buffer solution without polymer additives is used as the sample solution in the FASS scheme, the sample solution generates a larger EOF than the separation solution. These EOF differences for each region cause a laminar flow, broadening the electrophoresis band as shown in Fig. 4b. In contrast, with HEC dissolved in the sample buffer, DNA was concentrated in a flat band shape and no adsorption was observed.

The ratio of buffer concentration between the sample solution and separation solution, which is expressed by as the field enhancement factor, γ_{FASS}, affects electrophoretic resolution in the FASS scheme. Peak resolution increased as the γ_{FASS} increased, as shown in Fig. 5. When 0.2× TBE (γ_{FASS} = 4) was used as the separation solution, all peaks could be distinguished. Peak resolution increased with 0.5× TBE (γ_{FASS} = 10), but longer DNA fragments were not well-separated. When more than 5× TBE was used, 100-bp DNA ladder could be separated with sufficient resolution to distinguish the bands using computational methods. Since the FASS scheme concentrates the sample, electrophoretic bands can be narrow, and the sample can be separated faster than with the use of a conventional cross-injection scheme. Signal enhancement should be evaluated between signals of sample solution and separated samples at a detection point. As shown in Fig. 5, the signals of the separated sample at the detection point did not increase in the same manner as γ_{FASS}. Since the concentrated electrophoretic band became wider during separation due to dispersion, signal peak heights showed similar values for all buffer systems. The calculated sample concentration value from separated DNA signals was approximately 40 times for all buffer systems and DNA fragments.

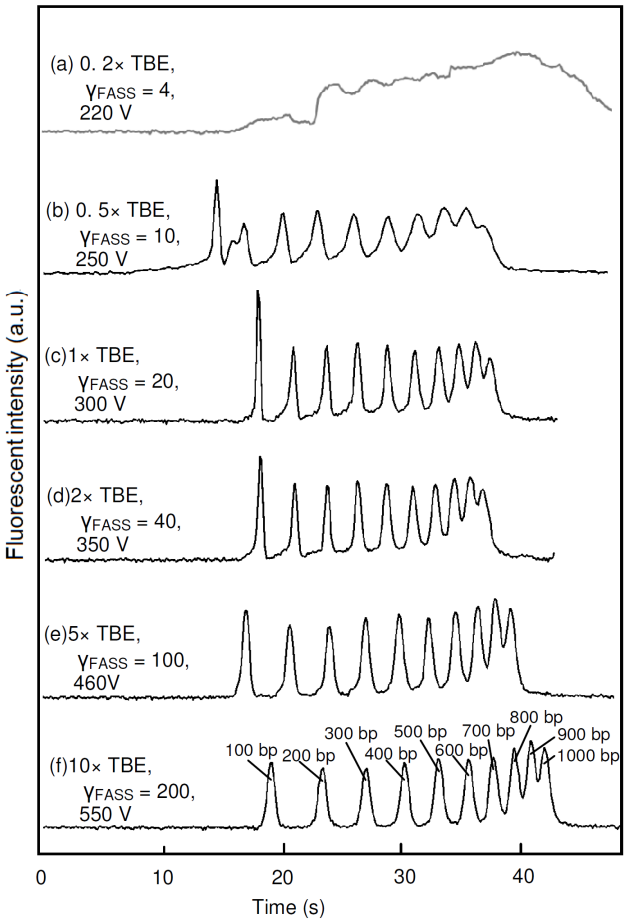

Figure 5. Comparison of electropherograms with different field enhancement factors, γ_{FASS} (the buffer concentration ratio between the sample solution and the separation solution). Concentrations of separation solution buffers, γ_{FASS}, and applied voltages are indicated. Voltages were adjusted such that samples would have similar electrophoretic mobility.

CONCLUSION

By adapting a partial barrier structure for a stop valve on the bottom of a microchannel, the partial barrier structure size on the side of a channel was successfully minimized. The minimized stop-valve structure was applied to electrophoretic sample plug formation. Since the sample plug was concentrated in a flat form after injection into the separation channel using the FASS scheme, high-separation resolution was achieved in a short period of time and separation channel length. Although approximately 2 min were required for liquid introduction into a 12-mm length channel, a 1.8-mm channel length is sufficient for separation. Therefore, the total operation time, including liquid introduction and electrophoresis, can be less than 2 min. This system is simple because SSCE can be operated using simple processes and electrical equipment.

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