PDMS MICROCHIP ELECTROPHORESIS WITH HIGH SEPARATION EFFICIENCY BY SIMPLE AND QUICK MODIFICATION OF PHOSPHOLIPID POLYMER

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ABSTRACT

This paper reports the high separation efficiency zone-electrophoresis of proteins in poly(dimethylsiloxane) (PDMS) microchip coated with the physically adsorbed phospholipid polymer, poly(2-methacryloyloxyethyl phosphorylcholine (MPC)-*co*-3-(methacryloyloxy)propyltris(trimethylsiloxy)silane (MPTSSi)) (PMMSi), using the simple and quick dip coating method. High suppression of protein adsorption and reduction of electroosmotic flow (EOF) were achieved by increasing hydrophilicity, and decreasing the surface ζ-potential of PDMS microchannels. Furthermore, proteins were completely separated within 30 seconds. It was confirmed that the durable and reproducible membrane of PMMSi could be formed on the PDMS surface, and it was applied for microchip electrophoresis (MCE) with high separation efficiency.

KEYWORDS

Microchip electrophoresis, PDMS, Phospholipid polymer, Surface modification.

INTRODUCTION

Electrophoresis is an effective technique for separation of DNA and proteins. In particular, microchip electrophoresis (MCE) has been popular because it can quickly analyze with small sample volumes and simultaneously diagnose several samples. Poly(dimethylsiloxane) (PDMS) has been widely used for polymeric material of microchip because of its excellent biocompatibility, chemical stability, optical transparency, non-toxicity, and ease of fabrication.

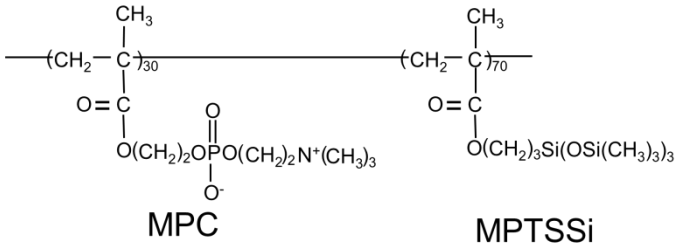

Figure 1. Chemical structure of PMMSi30.

However, the surface of PDMS is hydrophobic in its natural state, which leads to the adsorption of hydrophobic and biological samples such as proteins, peptides and DNA. The surface of PDMS also has negative charges, which cause adsorption of cationic samples through electrostatic interaction and generate unstable and strong electroosmotic flow (EOF). These factors are the common causes of deterioration in separation performance and detection sensitivity of MCE. The surface modification of PDMS is required to overcome these problems. However, there have not been reports on PDMS modification methods which contain all the ideal features for suppressing protein adsorption: the reduction of EOF, high durability, high reproducibility, simplicity and quickness of implementation. Simple and quick methods are required for the mass production of surface-modified PDMS microchip devices. Therefore, we aimed at the development of PDMS MCE with high separation efficiency by simple and quick modification of a phospholipid polymer. Phospholipid polymer has a phosphorylcholine group in the polymer side chains and it is one of the well-known hydrophilic and anti-fouling materials [1].

In this research, we developed the optimal molecular design of an amphiphilic phospholipid polymer composed of the phosphorylcholine moiety for decreasing the protein adsorption and the siloxane moiety for modifying a PMDS surface by use of polar solvents. Figure 1 shows the chemical structure of synthesized PMMSi30. PMMSi30 is reported as a coating material for PDMS using a polar solvent [2]. We evaluated the properties of PDMS surface coated with PMMSi30 by wettability, ζ-potential, and the coherent stability between PMMSi30 and PDMS. We confirmed the suppression of protein adsorption and reduction of EOF in PDMS microchips coated with PMMSi30. Electrophoresis of uranine was demonstrated using PMMSi30-coated PDMS microchip with high repeatability and reproducibility. Separation of two proteins (BSA and insulin) was also investigated with and without the surface modification. Results indicated that proteins were completely separated in the PMMSi30-coated PDMS microchip, which performed with high separation efficiency.

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EXPERIMENT

PMMSi30 was synthesized by the typical radical polymerization (MPC : MPTSSi = 3 : 7) (Figure 1). PMMSi30 was dissolved in ethanol at 30 mg/mL and modified by simply dipping PDMS elastomer in the polymer solution for 10 min and drying in the atmosphere.

To investigate the hydrophilicity of PDMS elastomer coated with each polymer, static contact angle of air bubble in water was measured. In this system, the contact angle of a hydrophobic surface becomes low, while that of a hydrophilic surface becomes large.

The air contact angle of PMMSi30-coated PDMS surfaces changed to approximately 150° from 80° of the native PDMS surface. Thus, we were able to confirm that the surface of PDMS elastomer converted from hydrophobic to hydrophilic by means of this simple and quick physical modification method.

ATR-FTIR spectra of bare PDMS and PDMS coated with PMMSi30 surfaces was obtained to determine whether the PMMSi30 was successfully modified on PDMS surface or not. As shown in Figure 3, ATR-FTIR result of the bare PDMS elastomer exhibited no absorption around 1720 cm^{-1} (C=O peak), while results of PDMS elastomers coated with PMMSi30 had 1720 cm^{-1} peaks. This was an indication that the polymer membrane was successfully formed on the PDMS elastomer. The presence of polymer membrane on PDMS surface also indicated its stability in water because PDMS elastomers coated with PMMSi30 had aged in water for 1 h.

The surface ζ-potential of PDMS elastomer coated with PMMSi30 was measured in 10 mM NaCl solution condition using an electrophoretic light-scattering spectrophotometer. PDMS elastomer of dimensions 30 × 30 × 2 mm^3 was used. The measurement was taken 3 times per sample. The ζ-potential of the PDMS surface changed from -31.3 ± 2.1 mV to -5.4 ± 2.3 mV after PMMSi30 coating. These results suggested that coating with PMMSi would reduce the EOF within the PDMS microchannel.

For modification of PDMS microchannel, PMMSi30 solution was injected through the inlet hole into the microchannel until it was filled. After 10 min, the polymer solution was pumped out from the microchannel. The microchannel was then dried under vacuum for 30 min. The dried samples were aged in a buffer for 30 min before taking measurements. The microchannel (Figure 2a) was filled with the protein solution (4.5 mg/mL FITC-labeled BSA in PBS) and aged for 30 min. Next, the microchannel was washed with fresh PBS to remove the protein solution. Then the microchannel was naturally dried in a clean box. Finally, the microchannel was observed using a fluorescence microscope at an exposure level of 1/3.5 s. Figure 4 shows the relative fluorescence intensity of microchannel after the protein adsorption test using the FITC-labeled BSA solution. As expected, compared with bare PDMS that collected significant amount of proteins, the relative intensity of the PDMS coated with PMMSi30 was significantly decreased almost to the background level. Thus, we confirmed that nonspecific protein adsorption can be effectively suppressed by PMMSi30 coating.

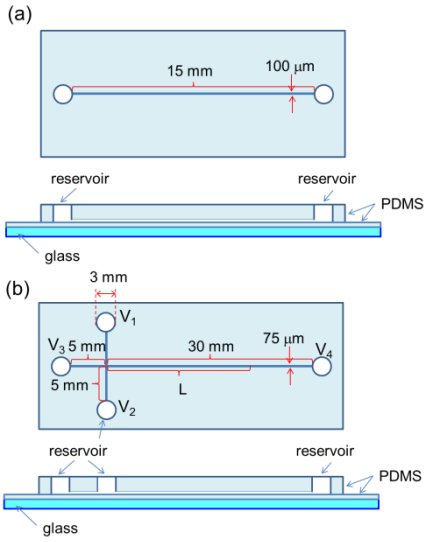

Figure 2. The schematics of PDMS microchips for (a) protein adsorption test and EOF measurement and (b) electrophoresis. Height: 50 μm.

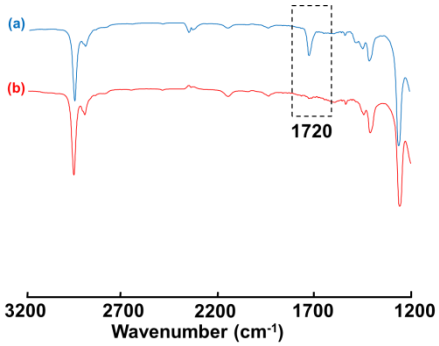

Figure 3. ATR-FTIR spectra of (a) PDMS coated with PMMSi30, and (b) bare PDMS.

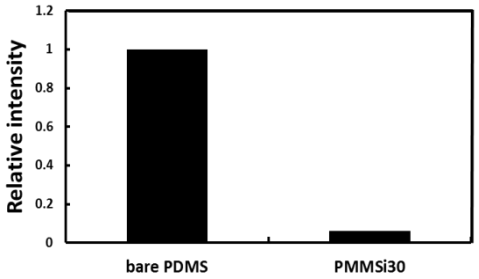

Figure 4. The relative fluorescence intensity of bare PDMS microchannel and PMMSi30-coated PDMS microchannel, obtained after the FITC-labeled BSA adsorption test.

In order to evaluate the durability of PMMSi30 membrane onto PDMS, MCE was performed in the PMMSi30-coated PDMS microchannel (Figure 2b) using uranine. In this experiment we used 10mM HEPES buffer (pH 7.0) solution. The measurement was repeated 10 times on the same PDMS microchip. The RSD of the durability of

PMMSi30 membrane in the MCE of uranine was 8.2% (n=10), indicating high durability of surface modification of PDMS, while bare PDMS chip could not be used for the duplicate measurement. The MCE of uranine on 5 pieces of PDMS microchip coated with PMMSi30 was performed to confirm the reproducibility of the polymer coating. The RSD was 4.1 %. We confirmed that the physical coating method of PMMSi30 on PDMS microchannel was a highly reproducible method for PDMS surface modification. EOF mobility was also calculated by the MCE of uranine. In this measurement, EOF mobility, μ_{eof}, was calculated according to the following equation *(Eq. 1)*.

$$\mu_{eof} = 2.9 \times 10^{-4} - \frac{l\,L}{t\,V} \qquad (1)$$

2.9×10^{-4} is electrophoretic mobility of uranine. l is effective separation length. L is separation length. t is the migration time of uranine. V is electric potential of separation channel, respectively. The average value of EOF mobility of the PDMS microchannel coated with PMMSi30 was 1.4×10^{-4} $cm^2V^{-1}s^{-1}$. In bare PDMS microchannel, EOF mobility is 5.7×10^{-4} $cm^2V^{-1}s^{-1}$. Therefore, successful suppression of EOF mobility within PMMSi30-coated PDMS microchannel was confirmed.

The MCE of the mixture of two proteins was carried out using the microchannel (Figure 2b). Two proteins were BSA (MW = 44 kDa, pI = 4.7) and insulin (MW =6.0 kDa, pI = 5.3). 10mM HEPES buffer (pH 7.0) was used as the running buffer. The separation microchannel length was 30 mm and the effective separation length was 10 mm. The voltages, V_1, V_2, V_3, and V_4 were adjusted according to each measurement (Figure 5). As illustrated in Figure 5(a, b), two symmetric peaks were observed on the MCE of the PMMSi30-coated PDMS microchannel, while only one peak was detected for the bare PDMS microchannel. In addition, the peak broadening occurred with the bare PDMS microchannel. This is because the hydrophobic interaction between proteins and PDMS resulted in the nonspecific protein adsorption. Also, the unstable EOF mobility due to protein adsorption might be one of the factors that resulted in the detection of only one peak. We calculated the separation factors of the MCE of the PMMS30-coated PDMS. Plate numbers were 2.0×10^3 for BSA and 2.3×10^3 for insulin. R_S (resolution) was 3.8. A_S (asymmetry factor) was 1.0 for BSA and 1.1 for insulin. Thus, it was confirmed that BSA and insulin were completely separated with high separation efficiency due to the suppression of protein adsorption and the reduction and stabilization of EOF. It was strongly suggested that zone electrophoresis in PDMS microchip coated with PMMSi30 has high repeatability on the same chip, high reproducibility of each microchip modification, and high separation efficiency of protein electrophoresis.

In conclusions, we developed the simple and quick modification method using PMMSi30. The high separation efficiency of the PMMSi30-coated PDMS-MCE was achieved by means of high suppression of nonspecific protein adsorption and reduction of EOF.

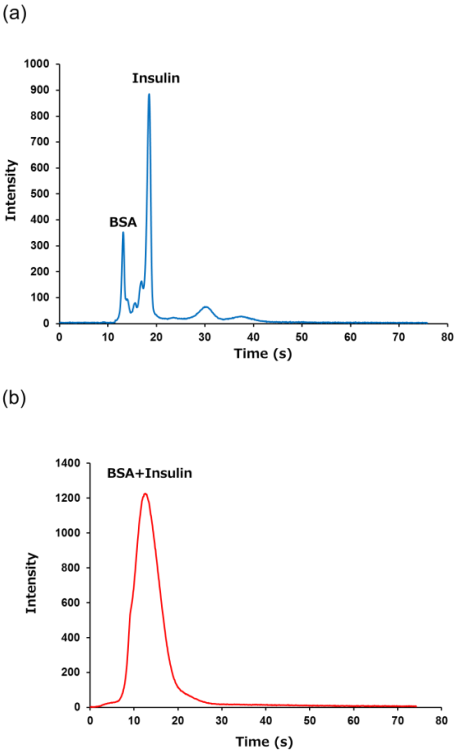

Figure 5. Electropherograms of 0.5 mg/mL BSA and insulin on (a) PMMSi30-coated PDMS microchip and (b) bare PDMS microchip. Conditions for (a): buffer: 10mM HEPES (pH 7.0); injection voltage: V_1= 0, V_2 = 0.5, V_3 = 0.1, V_4 = 0 [kV] for 60 s; separation voltage: V_1= 0.7, V_2 = 0.7, V_3 = 0, V_4 = 1.5 [kV]. Conditions for (b): buffer: 10mM HEPES (pH 7.0); injection voltage: V_1= 0.5, V_2 = 0, V_3 = 0.1, V_4 = 0 [kV] for 60 s; separation voltage: V_1= 0.7, V_2 = 0.7, V_3 = 1.5, V_4 = 0 [kV]

REFERENCES

[1] K. Ishihara, H. Nomura, T. Mihara, K. Kurita, Y. Iwasaki, N. Nakabayashi, *Why Do Phospholipid Polymers Reduce Protein Adsorption?*, J. Biomed. Mater. Res., 39, 323 (1998).

[2] J. H. Seo, T. Shibayama, M. Takai, K. Ishihara, *Quick and simple modification of poly(dimethylsiloxane) surface by optimized molecular design of the anti-biofouling phospholipid copolymer.*, Soft Matter, 7, 2968 (2011).

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