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Effects of grafting poly(ethylene oxide) on the amplification efficiency of a poly(dimethylsiloxane)-based flow-through PCR device

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ABSTRACT

The effects of grafting poly(ethylene oxide) (PEO) onto the surface of a microchannel on the amplification efficiency of a microfabricated device for polymerase chain reaction (PCR) were studied. The PCR device was composed of a poly(dimethylsiloxane) microchannel and a glass-heating chip. The PEO chains were grafted using neat silane or Pluronic® F127, and the presence of PEO was confirmed by water contact angle analysis and X-ray photoelectron spectroscopy. The surface treatments with neat silane and 10% (w/v) Pluronic® F127 resulted in an increase in the PCR amplification of a 298-bp DNA product by 2.2-fold and 3.9-fold, respectively, while 1.7-fold and 2.3-fold increases, respectively, were observed for a 1.1-kb DNA product. Both treatments could effectively enhance PCR efficiency even when DNA template concentration was decreased from 20 to 2 pg/μl. Our results indicated that these simple surface treatments could therefore be used routinely to enhance the performance of similar devices.

1. Introduction

Polymerase chain reaction (PCR) is the most widely used technique for DNA amplification. Different designs of miniaturized PCR devices have been developed, such as single chamber thermocyclers, array thermocyclers, and flow-through devices, because of their low reagent consumption, short reaction time, and portability [1,2]. In a flow-through device, PCR mixture passes through different temperature zones to achieve thermal cycling [3].

Early miniaturized devices were often fabricated in silicon or glass; however, the fabrication processes were usually expensive and cumbersome. Polymeric materials have become attractive alternatives because of their low cost and simple fabrication process, and poly(dimethylsiloxane) (PDMS) has received a lot of attention because it is chemically stable, optically transparent, and biocompatible [4]. A variety of microfluidic devices have been fabricated in PDMS using a set of techniques collectively called soft lithography [5]. Despite the advantages of PDMS, its hydrophobicity may cause problems such as difficulty in filling aqueous solution and adsorption of biological molecules; especially in miniaturized devices with high surface-to-volume ratios. A wide range of techniques, such as chemical vapor deposition, covalent modification, and formation of the polyelectrolyte multilayer, have been used to modify PDMS [4]. Two recent reports showed that protein adsorption was decreased significantly by grafting poly(ethylene oxide) (PEO) onto the surface of PDMS microchannel: (i) Sui et al. oxidized PDMS microchannel with acidic H_2O_2 solution followed by silanization using PEO-containing neat silane [6]. (ii) Boxshall et al. modified PDMS surface by adsorbing Pluronic® (a PEO–poly(propylene oxide) (PPO)–PEO tri-block copolymer) which employed the PPO block as a hydrophobic anchor [7].

In this work, we treated the PDMS microchannel of a flow-through PCR device with neat silane or Pluronic® F127 to determine if they could enhance the amplification efficiency. The amplification efficiency of microfluidic PCR device is often limited by interactions between the surface and the biomolecules in the PCR mixture, primarily due to increased surface-to-volume ratio [2]. The PCR device composed of a PDMS microchannel and a glass-heating chip with thin-film chromium resistive heaters was developed in our previous work [8]. We adopted the simple two-step PCR by performing annealing and extension at the same temperature [9]. The treated surfaces were characterized using water contact angle and X-ray photoelectron spectroscopy (XPS); the effects of the treatments on PCR efficiency were determined using gel electrophoresis.

2. Experimental

The fabrication process for the PCR device was modified from that in our previous work [8]. Two major differences were: (i) The sealing of the microchannel was achieved by oxygen plasma

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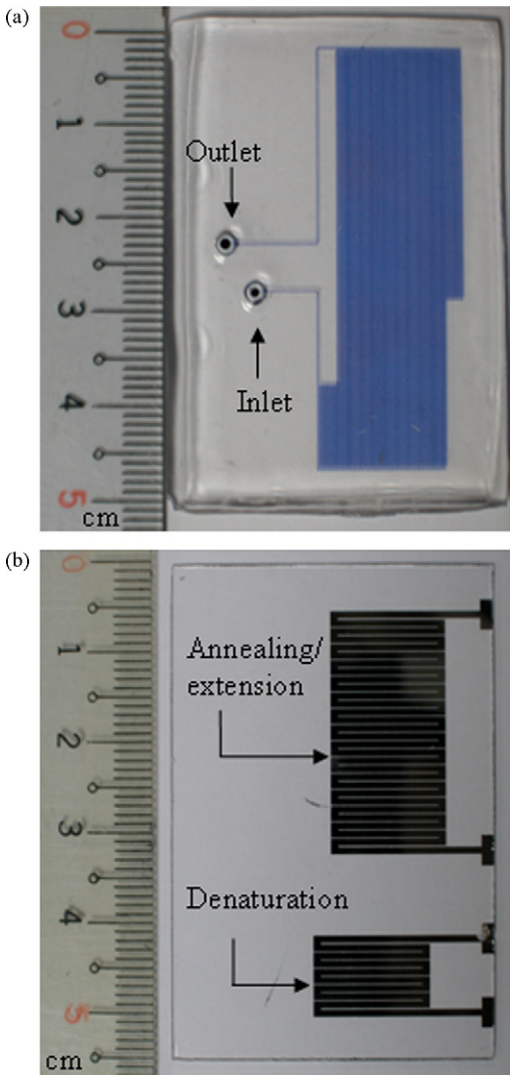

Fig. 1. Top views of the flow-through PCR device. (a) The PDMS microchannel, which was filled with 0.3% (w/v) methylene blue for visualization. The channel was 100 μm wide and 50 μm deep with a total length of 3.2 m allowing 30 cycles of the PCR reaction. (b) The glass-heating chip with thin-film chromium resistive heaters.

treatment (PDC-32G, Harrick Plasma) instead of a PDMS adhesion layer. (ii) The microchannel was not permanently attached to the glass-heating chip. The device is shown in Fig. 1.

The surface treatment was achieved by delivering neat silane (Gelest) or Pluronic® F127 (Sigma) into the PDMS microchannel at a flow rate of 0.3 μl/min with a syringe pump for overnight, followed by washing with 100 μl deionized water at a flow rate of 3 μl/min. Before the treatment with neat silane, the microchannel was first oxidized by flowing a mixture of H_2O/H_2O_2/HCl (volumetric ratio of 5:1:1) at 0.3 μl/min for 1 h, followed by washing with deionized water. All coating and washing steps were performed at room temperature. For water contact angle (goniometer G1, ERMA Optical Works) and XPS (ESCALAB 250, Thermo VG Scientific) analyses, 4 cm × 4 cm × 0.5 cm PDMS pieces were used and surface treatments were performed by immersion instead of flowing the reagents.

The PCR mixture containing 0.05 U/μl *Taq* polymerase, 100 μM dNTPs, 1 μM primers, and 20 pg/μl of the DNA template (a recombinant pET-23a(+) carrying the cDNA gene for *Trigonopsis variabilis* D-amino acid oxidase or a recombinant pET-26b(+) carrying the cDNA gene for *Bacillus natto* nattokinase) was delivered with a syringe pump. The lengths of the expected products amplified from recombinant pET-23a(+) and pET-26b(+) templates were 298 bp and 1.1 kb, respectively. The resistive heaters were connected to PID-controlled power supplies; the temperatures for the denaturation and annealing/extension heaters were set at 103 and 60 °C, respectively, which were optimized in preliminary experiments. Higher denaturation temperature was used because the actual temperature in the microchannel was lower than the temperature measured at the surface of the device [9]. The amplified product was analyzed on a 1.2% agarose gel and visualized by staining with ethidium bromide. The DNA band was quantified with Scion Image (www.scioncorp.com) software.

3. Results and discussion

The volumetric flow rate determines the residence time of the PCR mixture in each temperature zone and thus affects amplification efficiency. The effects of flow rates of 0.25, 0.5, 0.75, and 1 μl/min (the corresponding cycling times were 52, 26, 17, and 13 min, respectively) were determined. The amount of the 298-bp DNA product decreased as the flow rate increased. The highest flow rate (1 μl/min) resulted in no amplification, probably due to insufficient residence time. At flow rate of 0.75 μl/min (17-min cycling time), a clear product band was observed on the electrophoresis gel. The minimal 17-min cycling time required to produce observable amount of DNA product was comparable to those of similar devices [10,11]. By performing a negative control (PCR mixture without template) immediately after a successful sample run and a washing run, we demonstrated that cross contamination was minimal since no product band was observed.

The temporal change in the static water contact angle was determined immediately after treatment up to 22 d; samples were stored under ambient environment. The contact angle of untreated PDMS was stable at 104°, which was not too different from the reported 108° [12]. The contact angle decreased to 75°, 84°, 80°, and 84° immediately after treating with neat silane, 1%, 3%, and 10% (w/v) Pluronic® F127, respectively, suggesting the presence of the hydrophilic PEO chains. After 22 d, the contact angle gradually returned to 83°, 99°, 91°, and 90° for treatments with neat silane, 1%, 3%, and 10% Pluronic® F127, respectively. Hydrophobicity recovery for neat silane-modified PDMS has been reported [6]. The main mechanism has been attributed to the migration of low molecular weight chains from the bulk [4,13]. For treatments with Pluronic® F127, in addition to the migration of low molecular weight chains, desorption could also play a role in the recovery of hydrophobicity.

The low-resolution XPS spectrum of untreated PDMS exhibited an O 1s/C 1s photoemission ratio of 1.55. For 1% and 3% Pluronic® F127-treated PDMS, the O 1s/C 1s ratios did not change significantly; only the 10% treatment increased the ratio to 1.8. For neat silane-treated PDMS, the O 1s/C 1s ratio increased to 1.72. High-resolution C 1s XPS spectra further confirmed the presence of PEO (Fig. 2). For 10% Pluronic® F127 and neat silane-treated PDMS, new C 1s photoemission peaks centered at 286.5 eV were observed in the vicinity of the original Si-linked carbon centered at 284.6 eV. The new C 1s peak was identified as the O-linked carbon [14]. However, the C–O peak was not observed for 1% and 3% Pluronic® F127-treated PDMS.

Due to the gradual recovery of surface hydrophobicity, the effect of surface treatment on PCR efficiency was evaluated immediately after the treatment. Initial attempts to treat the PDMS microchannel in a static mode instead of a flowing mode failed because air gaps were developed at various locations of the microchannel after overnight incubation. The formation of air gaps may result from evaporation because PDMS is gas permeable. The effects of Pluronic® F127 treatments on PCR efficiency for the 298-bp DNA

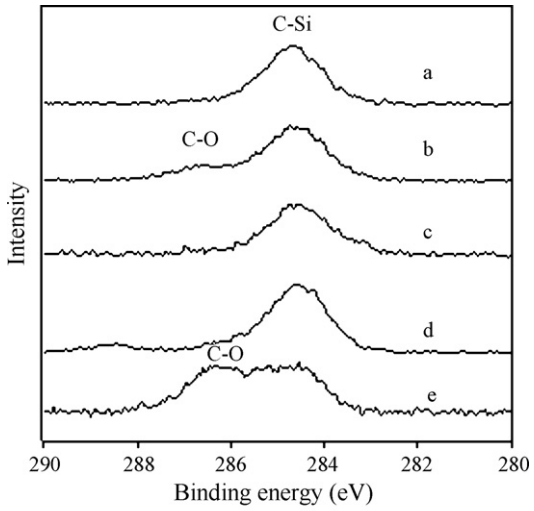

Fig. 2. High-resolution C 1s XPS spectra of PDMS. (a) Untreated, (b) neat silane-treated, (c–e) 1%, 3%, and 10% Pluronic® F127-treated, respectively. The C–O peaks at approximately 286.5 eV were observed for neat silane and 10% Pluronic® F127-treated PDMS.

product are shown in Fig. 3. The 1% Pluronic® F127 treatment had almost no effect. The fluorescence of the DNA product band increased by 3.1-fold and 3.9-fold for the 3% and 10% Pluronic® F127 treatments, respectively, compared with that from the untreated PDMS. In addition to the target DNA product, amplicons smaller than 100 bp were also observed. Further experiments demonstrated that these were most likely primer-dimers. Although the

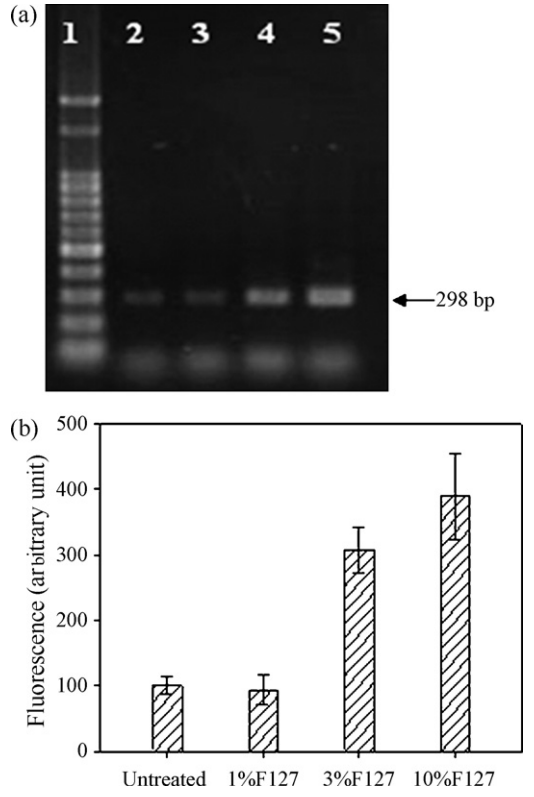

Fig. 3. Effects of the Pluronic® F127 treatments on PCR efficiency for the 298-bp DNA product. (a) Lane 1: DNA ladder; lanes 2–5 correspond to DNA products from the untreated, 1%, 3%, and 10% Pluronic® F127-treated PDMS, respectively. (b) Fluorescence of the product bands. The flow rate for PCR was 0.5 μl/min.

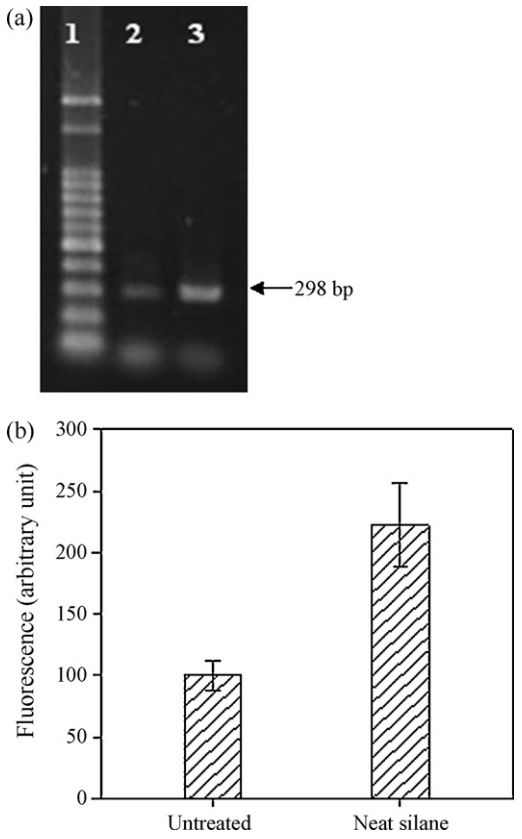

Fig. 4. Effect of the neat silane treatment on PCR efficiency for the 298-bp DNA product. (a) Lane 1: DNA ladder; lanes 2 and 3 correspond to DNA products from untreated and neat silane-treated PDMS, respectively. (b) Fluorescence of the product bands. The flow rate for PCR was 0.5 μl/min.

3% Pluronic® F127 treatment increased PCR efficiency, this was inconsistent with the result from XPS. The effect of the neat silane treatment on PCR efficiency for the same 298-bp product is shown in Fig. 4; the fluorescence of the DNA product band increased by 2.2-fold compared with that from the untreated PDMS. We also included a common surface passivation reagent, bovine serum albumin (BSA), for comparison [1]. After coating the surface with 10% (w/v) BSA under the same condition, the fluorescence of the 298-bp product band increased by 1.3-fold compared with that from the untreated PDMS.

We selected the most effective Pluronic® F127 concentration (10%, w/v) and neat silane for further studies. To test the effects of these treatments on PCR efficiency for longer DNA product, a recombinant plasmid containing the cDNA gene for *B. natto* nattokinase along with the corresponding primers were used; the expected length of the amplified product was approximately 1.1 kb. The fluorescence of the 1.1-kb DNA product band increased by 1.7-fold and 2.3-fold after neat silane and 10% Pluronic® F127 treatments (Fig. 5), respectively, compared with that from the untreated PDMS. The results suggest that the treatments are also effective for longer DNA product. We also examined the effects of these treatments on PCR efficiency at low DNA template concentrations by diluting the template 10-fold (2 pg/μl) and 100-fold (0.2 pg/μl). In the control runs performed with a commercial thermocycler, the 10-fold dilution did not decrease the amplification efficiency. The 100-fold dilution resulted in 10% decrease of the fluorescence of the 298-bp DNA product, compared with that from the undiluted reaction. In untreated PCR device, both dilutions resulted in no amplification (Fig. 6, lanes 2 and 3), which could be partly explained

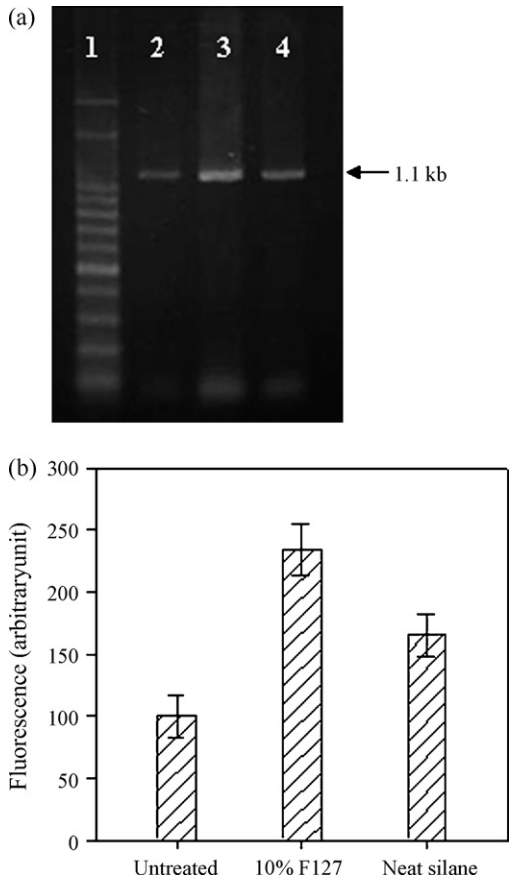

Fig. 5. Effect of the neat silane and 10% Pluronic® F127 treatments on PCR efficiency for the 1.1-kb DNA product. (a) Lane 1: DNA ladder; lanes 2–4 correspond to DNA products from untreated, 10% Pluronic® F127-treated, and neat silane-treated PDMS, respectively. (b) Fluorescence of the product bands. The flow rate for PCR was decreased to 0.25 μl/min due to longer DNA product length.

by the loss of DNA template at low concentration due to surface adsorption in a high surface-to-volume ratio microfluidic device. It has been proposed that the hydrophilic DNA molecule may exhibit some hydrophobicity through its bases, which may lead to the adsorption onto the hydrophobic surface of PDMS [15,16]. After neat silane treatment, the expected 298-bp product bands were observed for both dilutions (Fig. 6, lanes 4 and 5), but the fluorescence of the DNA product using 100-fold diluted template decreased by 85% compared with that from the 10-fold diluted reaction. After 10% Pluronic® F127 treatment, a clear product band was observed for the reaction using 10-fold diluted template (Fig. 6, lane 6), but there was still no amplification for the reaction using 100-fold diluted DNA template (Fig. 6, lane 7).

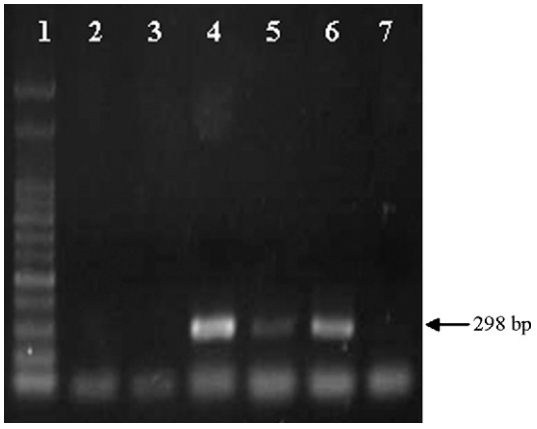

Fig. 6. Effect of the neat silane and 10% Pluronic® F127 treatment on PCR efficiency at low DNA template concentration. Lane 1: DNA ladder; lanes 2 and 3: no amplification was observed from untreated PDMS; lanes 4 and 5: DNA products from neat silane-treated PDMS; lanes 6 and 7: DNA products from 10% Pluronic® F127-treated PDMS. For lanes 2, 4, and 6, 2 pg/μl DNA template was used; for lanes 3, 5, and 7, 0.2 pg/μl DNA template was used. The flow rate for PCR was 0.5 μl/min.

The observed enhancement in PCR efficiency may result from a combination of several mechanisms. One possible mechanism might be a decrease in the adsorption of *Taq* polymerase on the surface of the microchannel because both neat silane and Pluronic® F127 could reduce the adsorption of proteins on PDMS [6,7]. The same mechanism was also proposed by others for their microfluidic PCR devices using a variety of surface modification techniques and fabrication substrates [17,18]. In addition to the surface passivation by these treatments, Xia et al. [19] reported that the presence of PEO may enhance the catalytic efficiency of *Taq* polymerase directly. The decreased adsorption of DNA template may also play a role in the observed enhancement based on the results using the diluted DNA template in this work. It should be emphasized the effects of these treatments on PCR efficiency can be different when more complex template such as genomic DNA or whole cell is used because these templates may interact with the surface of PDMS in a different manner.

From the temporal water contact angle analysis described previously, the gradual recovery of hydrophobicity after surface treatments may lead to a recurring adsorption of biological molecules which may result in decreased amplification efficiency. We tested the reusability of treated devices by performing successive runs using 2 pg/μl of the DNA template because such concentration was more sensitive to these treatments (no amplification unless the device was treated, Fig. 6, lane 2). Between runs, the PDMS microchannel was washed with 100 μl deionized water at a flow rate of 1.5 μl/min, and then dried with flowing air. For the neat silane treatment, the fluorescence of the 298-bp product band from the second run decreased by 42% compared with that from the first run, and no amplification was observed for the third run. Similar results were observed for the 10% Pluronic® F127 treatment. The rapid loss of amplification efficiency could be partly explained by the potential faster hydrophobicity recovery rate at elevated temperatures, it has been shown that the recovery half-times of hydrophobicity for corona discharge-treated PDMS decreased by at least five-fold when the storage temperature was increased from 25 to 100 °C [20]. For Pluronic® F127, the elevated temperature may also lead to a faster desorption.

4. Conclusions

For the 298-bp and 1.1-kb DNA products we tested, neat silane and 10% Pluronic® F127 treatments increased PCR efficiency by at least 1.7-fold. When the DNA template concentration was decreased from 20 to 2 pg/μl, both treatments still effectively enhanced PCR efficiency. In addition to enhancing amplification, the decreased surface hydrophobicity resulted from these treatments may also suppress bubble generation within the microchannel, a problem often encountered in this type of device [2,12]. Neat silane and 10% Pluronic® F127 treatments were comparable in terms of enhancing PCR efficiency under the conditions we tested; both methods were simple, especially the Pluronic® F127 treatment which was completed in a single step without prior oxidation. From the standpoint of device fabrication, the ability to perform the treatment after the microchannel is sealed may simplify the fabrication process. In this work, the surface modifi-

cation was achieved by flowing the reagent over the surface of the microchannel, it is possible to further increase PCR efficiency if the treatment is performed in a static mode, provided that the evaporation of reagent inside the microchannel is minimized. However, the rapid decay in PCR efficiency of treated device after repeated use renders these treatments more appropriate for the single-use devices.

Acknowledgements

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