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Surface modification of polydimethylsiloxane microfluidic chips by polyamidoamine dendrimers for amino acid separation

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ABSTRACT: In order to improve the hydrophilicity of polydimethylsiloxane (PDMS) microfluidic chips, a series of polyamidoamine (PAMAM) dendrimers were grafted onto the PDMS pretreated by oxygen plasma and silanized with γ-glycidoxypropyltrimethoxysilane (γ-GPS). Fourier transformed infrared absorption by total attenuated reflection (ATR-FTIR) and scanning electronic microscopy (SEM) showed that PAMAM dendrimers had been grafted on the PDMS surface and a dense and uniform coating was generated. The contact angle decreased from 108.1° to 31.8° compared with the unmodified PDMS, which indicated that the hydrophilicity of PDMS improved markedly. In the modified PDMS microfluidic chips, the EOF reduced obviously and the nonspecific adsorption between the amino acids and the microchannel surface was prevented effectively. The separation efficiency of arginine and phenylalanine were as high as 7.34×10^4 plates/m and 8.61×10^4 plates/m, respectively, and the resolution (R_S) was 4.41. Furthermore, the reproducibility of modified chips was also very excellent, which suggested they had good stability and long service life. © 2016 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2016**, *133*, 43580.

KEYWORDS: adsorption; dendrimers; grafting; hydrophilicity; microfluidics

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INTRODUCTION

During the last two decades, microfluidic devices have received much attention in the field of analytical chemistry because of high speed and efficiency, reduced sample consumption and analytical time, and even potential to be fabricated into high-density arrays to achieve high throughput.[1–5] Polydimethylsiloxane (PDMS) is one of the most popular materials in microfluidic devices for its advantages, such as cheap cost, large-scale manufacturing, chemical inertia, optical transparency, and biocompatibility.[6,7] Although PDMS has many merits, its hydrophobicity makes introducing aqueous solutions into the microchannels of PDMS-based devices difficult.[8] In addition, EOF is often unstable, and hydrophobic analytes can readily adsorb onto the PDMS surface interfering with analysis.[9] Therefore, it is often necessary to modify the surface of PDMS microfluidic chips for improving their hydrophilicity and inhibiting the absorption between analytes and PDMS.[10]

Generally, the surface modification of PDMS microfluidic chips includes two main approaches: (1) physical approach including physisorption of charged or amphiphilic hydrophilic polymers and copolymers,[11–14] as well as (2) chemical approach including self assembled monolayer and thick hydrophilic polymer coating.[15–20] Lee and Voros[21] demonstrated the coating of PLL-g-PEG on oxygen plasma treated PDMS surface and achieved excellent protein resistance. Wu *et al.*[22] modified the surface of PDMS microfluidic chips using self-assembled hydrophilic polymers for EOF inhibition and biopolymers separation. By now, the modified materials utilized for PDMS microfluidic chips are only limited to traditional linear polymers, such as polyacrylamide,[23] poly(ethylene glycol)[24–26] and poly(vinyl alcohol).[27,28] Although a lot of progress in traditional linear modified materials has been made, there are still some problems to resolve. Many modified materials are very difficult to coat on the surface of PDMS microfluidic chips due to rapidly increasing viscosity corresponding to a higher solution concentration. Moreover, the reproducibility of modified chips is poor because the linear polymers have no adequate functional groups to graft on the surface of PDMS, which lead to the coating unstable.

As a novel polymer, dendrimers have generated significant interest in the field of polymer materials due to their unique molecular structure and property.[29] One of their excellent properties is that they exhibit lower viscosity than the corresponding linear counterparts due to the lack of chain entanglement resulting from their highly branched molecular structure, so they are easily coated onto the surface of PDMS and generated integrated and uniform coating. Furthermore, dendrimers have plenty of

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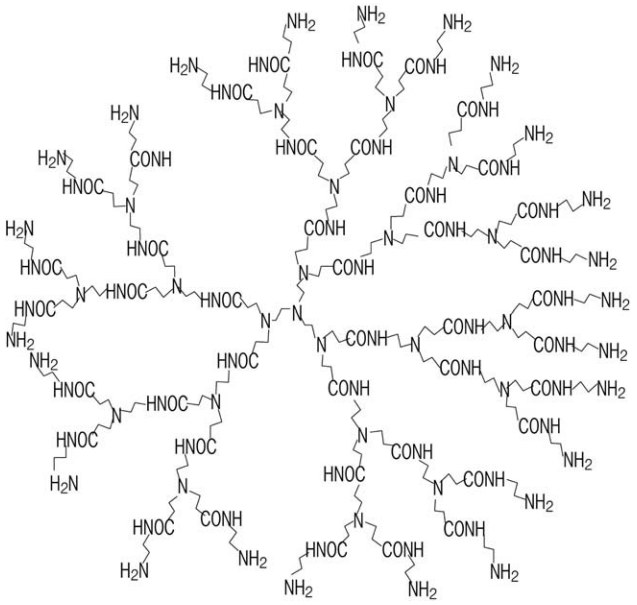


Figure 1. Molecular structure of PAMAM dendrimer used in this work.

reactive groups on their molecule surface that make them firmly bond onto the surface of PDMS and form a stable and reproducible coating. According to a review of the literature available to us, dendrimers have not been used for the modification of PDMS microfluidic chips. In this article, we describe a technique using hydrophilic polyamidoamine (PAMAM) dendrimers (Figure 1) via the induced graft polymerization to modify the surface of PDMS microfluidic chips for electrophoretic separation of amino acids.

EXPERIMENTAL

Materials

Sylgard 184 PDMS prepolymer and curing agents were obtained from Dow Corning (Midland, MI). The silicon template with protruding features was obtained from Northeastern University Analysis Centre for Scientific Research (Shenyang, Liaoning, China). Arginine, phenylalanine and fluorescein isothiocyanate (FITC) were purchased from Aladdin (Shanghai, China). The second generation (G2), third generation (G3), and fourth generation (G4) PAMAM dendrimers with tri(2-aminoethyl)amine as the core were synthesized and purified as described before.[30] Polyacrylamide were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). γ-Glycidoxypropyltrimethoxysilane (γ-GPS) were obtained from Shanghai Chemical Reagents (Shanghai, China). The deionized water used was from a Molresearcl 1005a water purification system (Molecular, Shanghai, China), The buffer solution used throughout the experiments was sodium borate buffer (5 m*M*, pH 9.0), which was filtered using 0.2-μm cellulose acetate filter.

Microchip Fabrication

Sylgard 184 PDMS prepolymer was mixed thoroughly with its curing agent at 10:1 (v/v), and then degassed by a vacuum pump. The mixture was cast onto the silicon template and cured at 70 °C for 2 h. After cooling to room temperature, the replica was peeled from the template, four holes were punched with a hole punch. Microfluidic channel pattern was shown in Figure 2. Similarly, using glass slide as a template, flat PDMS substrate (cover plate) was obtained according to the same steps. The PDMS substrate with microchannel and the cover plate were both cut into 2 cm × 5 cm pieces before use.

Oxygen Plasma Activation

The PDMS substrate with microchannel and the cover plate were treated by oxygen plasma generated in a DEM-451 reactive ion etcher (Anelva, Tokyo, Japan). The power of plasma was maintained at 80 W and the treatment time was kept for 1 min. Then, two parts were pasted together immediately for irreversible bonding.

After oxygen plasma treatment, the silanol groups were generated on the PDMS surface, which provided handles for subsequent chemical reactions.

Silane Treatment

After two parts were bonded together, the microchannel was flushed with 5% γ-GPS in carbinol for 30 min, and the remaining γ-GPS was removed by purging with nitrogen, then heated in 110 °C for 40 min. After the chip was cooled to room temperature, the microchannel was washed thoroughly with methanol and deionized water.

Grafting of PAMAM Dendrimers on PDMS Surface

About 5 mL of 10% PAMAM dendrimer in carbinol was passed through the microchannel after silane treatment for 30 min, and the remaining solution was removed by purging with nitrogen. Then, the chip was heated at 60 °C for 4 h in a gas chromatography oven. Finally, the microchannel was washed thoroughly with methanol and deionized water. The reaction scheme is shown in Scheme 1.

Characterization Measurements of PDMS Substrate

Fourier transformed infrared absorption by total attenuated reflection (ATR-FTIR) spectra of PDMS substrate on a wedged ZnSe crystal were recorded using a TENSOR 27 spectrometer with a DTGS detector (Bruker, Ettlingen, Germany). All spectra were obtained at 45° of incidence for 16 scans with a resolution of 4 cm^{-1} in the range of 500–4000 cm^{-1}.

The contact angle of PDMS substrates were measured by a NRL-100 automatic contact angle measuring instrument (Dataphysics, Filderstadt, Germany). At room temperature, 4 μL deionized water was placed onto the surface of PDMS substrates

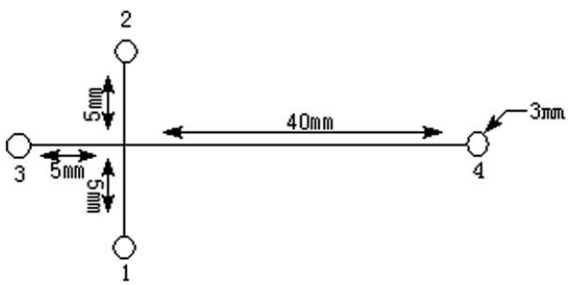
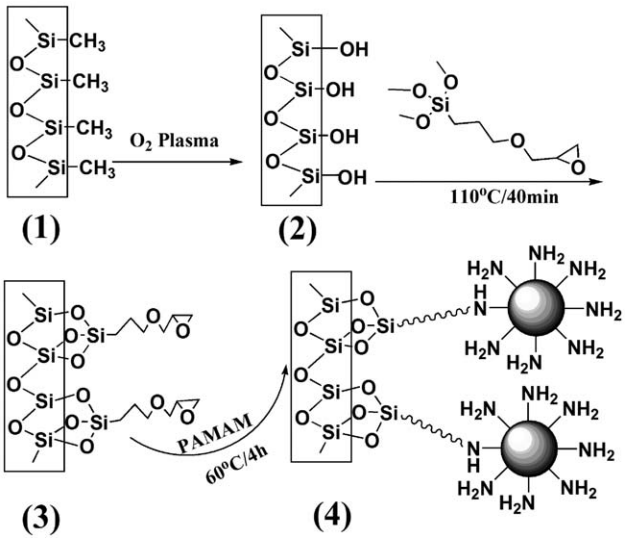


Figure 2. Schematic diagram of PDMS microfluidic chip used in this work: 1, sample reservoir; 2, sample waste reservoir; 3, buffer reservoir; 4, buffer waste reservoir. Microchannel: width: 46 μm, depth: 23 μm.



Scheme 1. Schematic of PAMAM dendrimers grafted onto surface of PDMS.

and then measured after standing 30 s. All data points were replicated three times and the average value was calculated.

The surface morphologies of PDMS substrates with enlarging 1000 and 3000 times were measured using a S-2500 scanning electronic microscopy (SEM) (Hitachi, Tokyo, Japan).

EOF Measurements

The EOF of PDMS microfluidic chips was determined using the current monitoring method.[31] The measurement procedure was as follows: Firstly, a buffer solution with a concentration of C_0 was pumped into the microchannel and detection reservoir using a syringe pump. After running for 5 min under a high pressure, the buffer solution in the sample reservoir was replace by the buffer solution with a concentration of 0.8 C_0. Then, +1000 V separation voltage was applied and the migration time (t) was determined. Electro-osmotic mobility, μ_{eof}, could be calculated from the eq. (1):

$$\mu_{eof} = (L/t)E^{-1} \quad (1)$$

In the eq. (1), L is the effective length of the microchannel and E is the electric field strength.

Amino Acid Electrophoresis

A homemade laser-induced fluorescence detection system was used to perform electrophoresis of the amino acid in PDMS microfluidic chip. Firstly, the microchannel and four reservoirs were filled with borax buffer using a syringe pump. Prior to separation, 40 μL of the amino acid solution (labeled by FITC) was introduced into reservoir 1 to replace the buffer. Then, a platinum electrode was inserted into each reservoir to provide electrical contact and voltages were applied to the reservoirs using XCDY intelligent high-voltage power supply for microfluidic chip (Chemical Technology Academy of Shandong, Jinan, Shandong, China). "Pinched" injection was used to introduce the sample into the microchannel. During injection, reservoir 1 was maintained at 0.5 kV and reservoir 2 was grounded. The injection time was kept for 15 s. During separation, reservoirs 1 and 2 were set at 0.2 kV, reservoir 3 was set at 1.0 kV, and reservoir 4 was grounded. These conditions were used for all of the electrophoretic experiments.

RESULTS AND DISCUSSION

Characterization Measurements of PDMS Substrate

The ATR-FTIR spectra of unmodified PDMS and modified PDMS substrates was shown Figure 3. The characteristic peaks at 2956, 1263, and 792 cm^{-1} were assigned to Si—CH_3. The Si—O—Si occurred at 1740 cm^{-1}. Compared with the unmodified PDMS, the modified PDMS clearly showed the characteristic bands of NH_2 at 3430 cm^{-1}, CH_2 at 2871 cm^{-1}, carbonyl at 1682 cm^{-1}, and CN at 1068 cm^{-1}, which demonstrated that PAMAM dendrimers had been grafted on the PDMS surface. The ATR-FTIR spectra of modified PDMS using G2, G3, and G4 were quite similar because the chemical structure of them resemble each other.

Contact angle can reflect the wettability of material surface. The smaller is the contact angle, the better is the hydrophilicity of materials. The contact angles of unmodified and modified PDMS substrates were measured and the results were shown in Table I. It was found that the contact angle of unmodified PDMS was 108.1°, while the data decreased considerably after modification. Especially for the PDMS substrates modified using G4, whose contact angle decreased to 31.8°, which indicated that the hydrophilicity of PDMS surface improved effectively. It was also found that the contact angle decreased more significantly with the generation increasing. The reason for this phenomenon was that the amine group amount of PAMAM dendrimers increased multiplied with the generation increasing, which provided more active sites for reacting and more PAMAM dendrimers were grafted onto the PDMS surface. In addition, the molecular weight also increased with the generation increasing. These two factors contributed to the

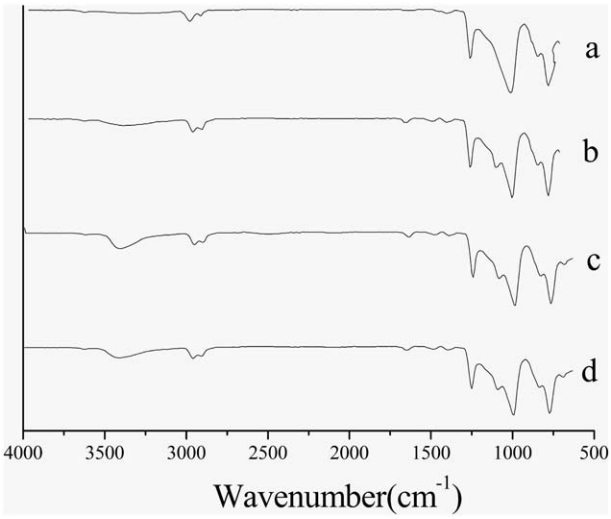


Figure 3. ATR-FTIR spectra of modified PDMS substrates: (a) unmodified PDMS; (b) G2 modified PDMS; (c) G3 modified PDMS; (d) G4 modified PDMS. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Table I. Contact Angle of PDMS Substrates

Sample	Unmodified PDMS	G2 modified PDMS	G3 modified PDMS	G4 modified PDMS
Contact angle	108.1°	47.2°	36.9°	31.8°

improvement of the coating thickness, so the hydrophilicity of PDMS surface was better.

In order to observe the surface morphology of PDMS after modification, the PDMS substrates modified using G4 were measured by SEM (Figure 4). It was shown that obvious change had taken place on the surface of modified PDMS. The surface of unmodified PDMS was very coarse. By contrast, the surface of modified PDMS became smooth and a dense and uniform coating was generated, which further indicated that the PAMAM dendrimers had been successfully grafted onto the PDMS surface and the native hydrophobic surface was fully transformed into a hydrophilic one.

EOF Measurements

The EOF is dependent on the magnitude of zeta potential across the double electric layer, which is determined by the charge density on the channel surface. Thus, the magnitude of the EOF reflects the property of the channel inner surface and the effect of surface treatment.[27] The EOF of unmodified and modified PDMS microfluidic chips was measured in the range of pH 3.0–11.0 and the results were show in Figure 5. For the unmodified chips, the EOF significantly increased with the increasing of pH, in contrast to the gradual increasing in the modified chips. The reason was that the coating of PAMAM dendrimers effectively shielded the ionization of the electriferous groups on the microchannel surface and the EOF was inhibited strongly. At the same pH, with the increasing of generation, the EOF of modified chips was inhibited more effectively, which resulted from more uniform and thicker coating shielding more electriferous groups ionized.

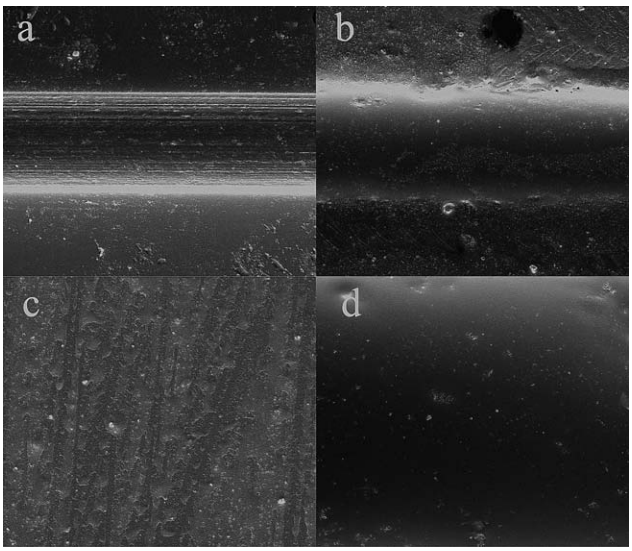


Figure 4. SEM pictures of G4 modified PDMS substrates: (a) unmodified PDMS after enlarging 1000 times; (b) modified PDMS after enlarging 1000 times; (c) unmodified PDMS after enlarging 3000 times; (d) modified PDMS after enlarging 3000 times.

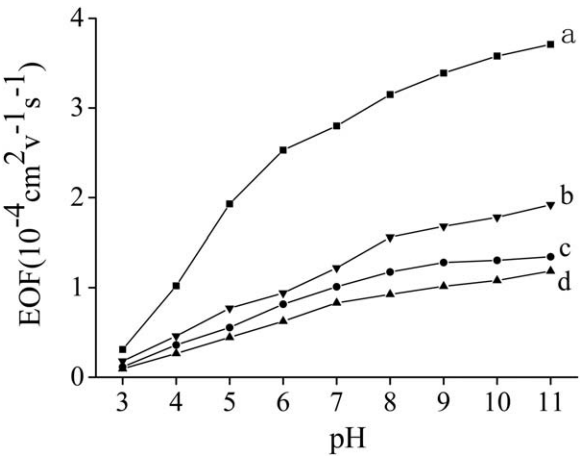


Figure 5. Effect of the buffer pH on EOF: (a) unmodified chip; (b) G2 modified chip; (c) G3 modified chip; (d,) G4 modified chip. Conditions: phosphate buffer C0 40 m*M*; Electric field strength, 400 V/cm; Running time was limited within 200 s.

Stability of the modified chips have an important influence on the analysis effect during electrophoresis. In this study, the microchannels of modified PDMS microfluidic chips were filled with 5 m*M* sodium borate buffer (pH 9.0), then the EOF within 20 days was continuously measured and the results are shown in Figure 6. It was found that the EOF of modified chips during this period had no significant change, which expressed that the coating of PAMAM dendrimers was very stable.

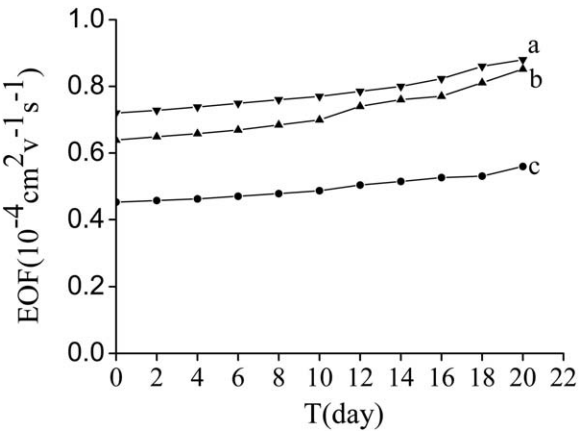


Figure 6. EOF of modified chip within 20 days: (a) G2 modified chip; (b) G3 modified chip; (c) G4 modified chip. Conditions are the same as in Figure 5.

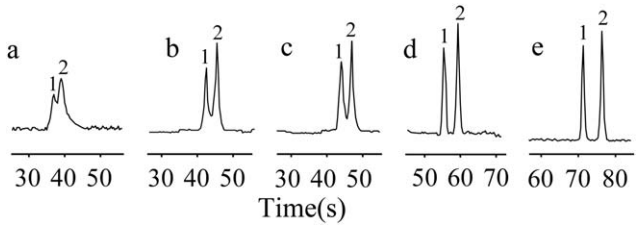


Figure 7. Electrophoresis figure of mixture of arginine on PDMS microfluidic chip: 1, arginine; 2, phenylalanine; a, unmodified chips; b, polyacrylamide modified chips; c, G2 modified chips; d, G3 modified chips; e, G4 modified chips. Separation conditions: 5 m*M* borate buffer, pH = 9.2; Effective separation channel length: 2 cm. Sample: Arginine (10 mg/L); Phenylalanine (10 mg/L); FITC (20 mg/L).

Amino Acid Electrophoresis

The performance of modified PDMS microfluidic chips were evaluated via separating the mixture of arginine and phenylalanine. For comparison, the modified PDMS microfluidic chips using traditional linear polyacrylamide were also choose to separate the two amino acids. The modification process was the same as PAMAM dendrimers. Figure 7 and Table II shows the electropherograms of the separation of arginine and phenylalanine in unmodified and modified chips.

Figure 7 shows that arginine and phenylalanine were not separated in the unmodified chips with a wider and tailing peak caused by serious absorption between the amino acids and the surface of the microchannel. At the same conditions, the polyacrylamide and PAMAM dendrimers modified chips could separate the two amino acids. But, the column efficiency of the PAMAM dendrimers modified chips was better than that of the polyacrylamide modified chips. The reason was that the PAMAM dendrimers had more functional groups than polyacrylamide on the molecular surface. As PAMAM dendrimers were grafted onto the surface of the chips, the formed coating was more integrated and stable and could effectively prevent the absorption. As a result, the column efficiency was higher. Moreover, the column efficiency of the PAMAM dendrimers modified chips became better with the generation increasing. Especially for G4 modified chips, the column efficiency for arginine and phenylalanine were as high as 7.34×10^4 plates/m and 8.61×10^4 plates/m, and resolutions (R_S) was 4.41. It was also observed that the migration time extended with the generation increasing. This is because that the EOF decreased more obviously for higher generation, which led to the migration rate of the analytes becoming slower.

Table II. Column Efficiency and Migration Time of Unmodified and Modified Chips

	Plates × 10^4		
Chips	Arginine	Phenylalanine	R_s
Unmodified chips	0.87	1.26	0.24
Polyacrylamide modified chips	1.93	2.36	0.91
G2 modified chips	2.21	2.54	0.99
G3 modified chips	3.67	6.59	2.71
G4 modified chips	7.34	8.61	4.41

Table III. Reproducibility of the Migration Time and Column Efficiency for the Modified Chips (RSD %)

	Arginine		Phenylalanine	
Chips	Time/s	Plates/ ×104	Time/s	Plates/ ×104
Polyacrylamide modified chips	5.17	5.32	4.92	4.61
G2 modified chips	3.83	3.91	3.75	3.82
G3 modified chips	3.11	3.48	2.83	3.16
G4 modified chips	2.09	2.76	1.42	2.09

Reproducibility

The reproducibility of the polyacrylamide and PAMAM dendrimers modified chips was determined by consecutive running ($n = 10$) (Table III). The results showed that the RSD of migration time and column efficiency were less than 3.83 and 3.91%, respectively, for the PAMAM dendrimers modified chips. The RSD values for the migration time and column efficiency of the PAMAM dendrimers modified chips were lower than those of the polyacrylamide modified chips, which indicated that the former possessed more excellent reproducibility than the latter.

CONCLUSIONS

PAMAM dendrimers were successfully grafted onto the surface of PDMS microfluidic chips and the formed hydrophilic coating effectively inhibited the EOF and the nonspecific adsorption between the amino acids and the surface of microchannel. Arginine and phenylalanine were successfully separated with a highly efficient in the PAMAM dendrimers modified chips. Furthermore, their reproducibility was also excellent. Based on the special molecular structure of PAMAM dendrimers, we believe that they not only can be used for the modification of PDMS substrate, but also can be applied in a much broader sense to other substrates for chips, such as glass, silicon, or other polymer materials.

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D.L. performed the majority of the experiments and wrote the article. Q.Z. performed the electrophoretic separation experiment, D.S. assisted with the writing of the article (especially image preparation).

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