NOVEL SURFACE MODIFICATION METHODS AND SURFACE PROPERTY ANALYSIS FOR SEPARATION OF DNA BIO-MOLECULES USING CAPILLARY ELECTROPHORESIS

Che-Hsin Lin[1], Lung-Ming Fu[2], Kuo-Hoong Lee[3], Ruey-Jen Yang[3], Gwo-Bin Lee[3]

[1]Department of Mechanical and Electro-mechanical Engineering, National Sun Yat-Sen University, [2]Department of Materials Engineering, National Pingtung University of Science and Technology, [3]Department of Engineering Science, National Cheng Kung University, Taiwan, ROC

ABSTRACT

This paper presents systematic investigation on the surface properties of electroosmotic flows (EOF) inside microchannels for quartz, glass and PDMS based materials. Two novel methods to modify the surface properties of glass-based microchannels for capillary electrophoresis (CE) are developed. Instead of using complicated and time-consuming chemical silanization procedures for surface modification of the CE channels, two simple and reliable methods utilizing organic-based spin-on-glass (SOG) and water-soluble acrylic resin are reported, providing a fast and batch process for surface modification of glass-based CE channels. The proposed methods are evaluated using separation of ΦX-174 DNA makers. Experimental data show that separation efficiency is greatly improved. In addition, long-term stability of the SOG coating is also verified in this study.

Keywords: surface modification, zeta potential, spin-on-glass, electroosmotic flow

1. INTRODUCTION

The development of MEMS technology has made a substantial impact on bio-analytical detection and open up a new field of interest commonly called "Lab-on-a-chip" within the past decade. For example, chip-based capillary electrophoresis has been used for rapid detection of specific DNA fragments for specific diseases. However, two common problems occurred for such a detection scheme, including electroosmotic flows (EOF) and the interactions between analytes and channel walls during sample separation. For many electrophoresis applications, reduction of the electroosmotic effect to diminish the bulk sample flow is preferred.

Typically, shielding of the silano groups exposed on the surface of silica-based channels is a common solution for these problems. This method was first demonstrated by Hjertén [1] in 1985 using a polymer layer (polyacrylamide, PAA) covering the capillary wall. However, PAA-coated silica surface has a pretty limited lifetime at an alkali solution due to alkali hydrolysis of the –Si-O-Si- bond [2].

In this study, novel approaches to coat the microchannels utilizing commercially-available organic-based spin-on-glass and acrylic resin were developed for silica-based channels. The time and cost for the surface modification could be dramatically reduced using the developed methods. In addition, stability of the zeta potential for PDMS microchannels treated with oxygen plasma was also evaluated. At last, ΦX-174 DNA markers were used to test the performance of the proposed coating layers.

2. MATERIALS AND METHODS

Micro CE chips (36 μm deep) with a 40-mm long separation channel were first fabricated using soda-lime glass [3], quartz and PDMS. The chips were treated using various kinds of surface modification methods prior to testing. The conditions are listed in Table 1. The SOG (200F) used in this study was acquired from Filmtronics, USA. PDMS chips were fabricated using PDMS elastomer (PEM-10, UCT, Inc., USA). Induced oxygen plasma was used to modify the surface properties of PDMS for 5 min. A 10^{-4} M Rhodamine B in a sodium borate buffer (pH=9.2) was used for CE testing and subsequent measurement of the electroosmotic velocity. The electric fields for measuring the EOF velocities are 400 V/cm and 200 V/cm for glass-based and PDMS microchannels, respectively.

3. RESULTS AND DISCUSSION

Figure 1 shows the measured electrokinetic mobilities for various kinds of glass-based microchannels. Results show the SOG and acrylic resin coating could dramatically reduce the electroosmotic effect in the CE channel. The SOG-coated surfaces have lower zeta potentials due to the shielding of the silanol groups. Note that the acrylic resin layer could provide a positive EOF (flows direct to the high electric field). HCl and NaOH solution washing will also refresh the silanol group on the glass-based channel and accordingly increase the EOF velocity significantly. Figure 2 shows the time dependency of the zeta potentials for PDMS microchannels stored in air and in DI water after oxygen plasma treatment. The generated silanol groups on the PDMS surface could increase the mobility of the buffer after O_2 plasma treatment. However, the silanol groups will decay with the time. It is also noticed that filling the channels with DI water could preserve the silanol groups in a period time

Figure 3 shows the cross section views of the coated SOG layer (Fig. 3a) and acrylic resin layer (Fig. 3b). The thickness for the coating was about 157 nm and 200 nm, respectively. Both of the coated layers could completely cover the glass surface yet the SOG layer could provide better surface smoothness, resulting in better separation efficiency. All 11 peaks for ϕX-174 DNA (Hae III digested) could be separated with a high efficiency (Fig. 4). The chemical properties of SOG coating layer is stable and can remain its properties without delicate storage condition such that storage cost can be reduced. The long-term surface stability was also evaluated by separating ϕX-174 DNA ladders (Fig. 5). The chip was tested 45 days after the surface coating and the chip was

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stored just in a clean incubator. The microchip coated with acrylic resin could also separate DNA samples successfully. The positive zeta potential of the acrylic resin even resulted in a faster separation speed. However, the zeta potential of the acrylic resin might change with time. Further studies for the phenomenon are undergoing.

4. CONCLUSIONS

We have demonstrated two novel methods for surface modification of glass-based microchannels with nano-scale coating layers. EOF velocity of the coated surface can be reduced significantly and even reverse flow direction can be achieved. The performance of the proposed methods could meet the requirement of rapid mass production since no time-consuming chemical process was used. Electroosmotic mobilities for various kinds of surfaces were also measured including quartz, glass and PDMS based materials. The performance of the proposed methods for surface modification and the long-term stability of SOG coating layer were also confirmed by separating standard DNA markers. The proposed method could modify microfluidic chips in a rapid and reliable way.

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Table 1. Velocities of the electroosmotic flow and the values of the calculated zeta potentials.

Material		Condition	EOF velocity (mm/s)	Zeta potential $-\zeta$ (mV)
Glass	A	Glass without cleaning	2.22	79.93
	B	Clean with NaOH and DI water for 5 min	2.86	102.77
	C	Clean with HCl, NaOH and DI water for 5 min respectively	3.08	110.68
	D	Quartz substrate with type C cleaning	3.08	110.68
	E	SOG coating	1.14	40.85
	F	Acrylic resin coating	-0.49	-17.76
PDMS	G	30 min after O_2 plasma treatment (in air / in DI water)	0.05 / 0.085	35.97 / 59.97

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H	1 hr after O$_2$ plasma treatment (in air / in DI water)	0.05 / 0.085	35.97 / 59.97
I	2 hr after O$_2$ plasma treatment (in air / in DI water)	0.04 / 0.05	27.97 / 35.97
J	4 hr after O$_2$ plasma treatment (in air / in DI water)	0.0015 / 0.05	21.15 / 35.97
K	24 hr after O$_2$ plasma treatment (in air / in DI water)	0.01 / 0.028	10.58 / 19.99
L	48 hr after O$_2$ plasma treatment (in air / in DI water)	0.005 / 0.007	5.15 / 5.29
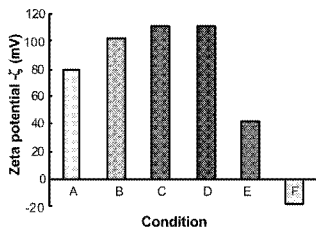
Fig. 1 Measured zeta potentials for glass-based microchannels with various kinds of treatments.
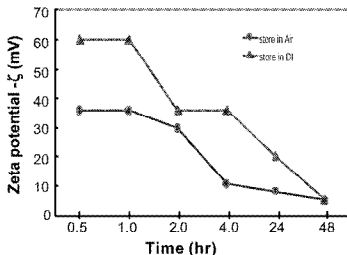
Fig. 2 Time dependency of the zeta potentials for PDMS microchannels stored in air and in DI water.
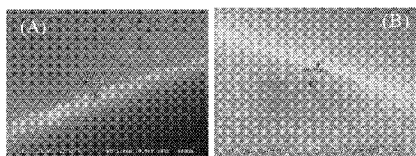

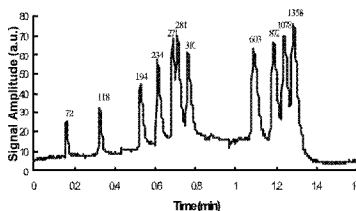Fig. 3 SEM cross-section images of the coated SOG layer (A) and acrylic resin layer (B).

Fig. 4 Electropherogram of φX-174 DNA ladders for SOG-coated micro CE chips. The experiment was performed right after the surface modification.
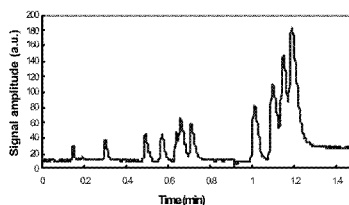

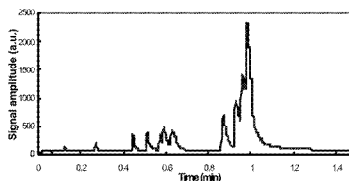Fig. 5 Electropherogram of φX-174 DNA ladders for SOG-coated micro CE chips (45 days after the surface modification).

Fig. 6 Electropherogram of φX-174 DNA ladders for acrylic-resin-coated micro CE chips. The experiment was performed right after the surface modification.

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