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Analysis of double-stranded DNA by microchip capillary electrophoresis using polymer solutions containing gold nanoparticles

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Abstract

The impact of gold nanoparticles (GNPs) on the microchip electrophoretic separation of double-stranded (ds) DNA using poly(ethylene oxide) (PEO) is described. Coating of the 75-μm separation channel on a poly(methyl methacrylate) (PMMA) plate in sequence with poly(vinyl pyrrolidone), PEO, and 13-nm GNPs is effective to improve reproducibility and resolution. In this study, we have also found that adding 13-nm GNPs to 1.5% PEO is extremely important to achieve high resolution and reproducibility for DNA separation. In terms of the stability of the GNPs, 100 m*M* glycine–citrate buffer at pH 9.2 is a good buffer system for preparing 1.5% PEO. The separation of DNA markers V and VI ranging in size from 8 to 2176 base pairs has been demonstrated using the three-layer-coated PMMA microdevice filled with 1.5% PEO containing the GNPs. Using these conditions, the analysis of the polymerase chain reaction products of UGT1A7 was complete in 7 min, with the relative standard deviation values of the peak heights and migration times less than 2.3% and 2.0%, respectively. In conjunction with stepwise changes of the concentrations of ethidium bromide (0.5 and 5 μg/ml), this method allows improved resolution and sensitivity for DNA markers V and VI.

Keywords: Gold nanoparticles; Chip technology; Microfluidics; DNA

1. Introduction

Capillary electrophoresis (CE) and microchip CE have proved powerful for DNA analysis because they offer great advantages over conventional slab gel electrophoresis, including high resolving power, rapidity, minute sample requirement, and ease of automation [1–5]. A sieving matrix providing high resolving ability is essential for DNA analysis because it determines both the DNA migration behavior and resolution. Owing to low viscosity, ease of preparation, and flexibility, a number of linear polymer solutions, including linear poly(acrylamide) (LPA), poly(ethylene oxide) (PEO), cellulose and its derivatives, and poly(vinyl pyrrolidone) (PVP) are common sieving matrices for the analysis of polymerase chain reaction (PCR) products, DNA sequencing, as well as diagnostic and forensic applications [6–18]. To achieve reproducibility and high resolution, coating of the capillary wall with inert molecules such as polymers is essential because it prevents the variation of electroosmotic flow (EOF) and minimizes the interactions with DNA. Hydroxyethylcellulose (HEC) [6], polydimethylacrylamide [8,12,16], PVP [9,11,15], LPA [13], and PEO [14]

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possess dynamic coating ability and are also used as coating matrices. It is widely believed that dynamic coating is through interactions such as hydrogen bonding, electrostatic, and hydrophobic patches between the capillary inner wall and the polymer molecules [17,18].

The separation channels in microchip CE have been fabricated in a variety of substrates, including glass, quartz, and polymer substrates [19–24]. Of these, polymer substrates such as poly(methyl methacrylate) (PMMA) [23,25] and polycarbonate (PC) [26] are particularly interesting because they offer a greater potential for making disposable devices on a cost-effective basis by printing, laser ablation, and plasma etching [24–26]. Although we have demonstrated improvements in resolution and sensitivity for PCR products using a bubble cell fabricated in a PVP-coated PMMA plate, the method is not promising for DNA sequencing in terms of efficiency [27]. This is mainly due to a poor dynamic coating, because the surface of PMMA (ester functional groups) is not hydrophilic and its negative charge density is low. To overcome this shortage, coating of PMMA channels through covalent bonding with chemicals has been proposed [28]. However, covalent bonding generally suffers from the need for a tedious coating process and a limited lifetime. Thus, it is our goal to develop a simple method to modify the surface of PMMA for DNA separation.

In a previous study, we found that DNA separations using a capillary coated with PVP and filled with low-viscosity PEO solutions (e.g. 0.2%) containing gold nanoparticles (GNPs) provide the advantages of reproducibility, high resolution and rapidity [29]. GNP-coated capillaries and microfabricated channels have been employed to enhance the separation efficiency for small isomers in CE and microchip CE [30,31]. The authors have suggested that the EOF changed due to adsorption of the GNPs on the capillary wall and the electrophoretic mobility of the solutes varied once they were interacted with the GNPs. Based on these results, we tested DNA separations on microfabricated CE channels coated with PVP, PEO, and 13-nm GNPs. We found that PEO prepared in a buffer composed of glycine, citrate, GNPs, and ethidium bromide (EtBr), pH 9.2, is proper in terms of reproducibility, speed, and resolution. To further optimize sensitivity and resolution without a great compensation for the loss of speed, a stepwise technique based on changes in EtBr concentration was applied.

2. Experimental

2.1. Equipment

The basic design of the separation system is similar to a previous one [27]. Briefly, a high-voltage power supply from Bertan (Hicksville, NY, USA) was used to drive electrophoresis. The entire detection system was enclosed in a black box with a high-voltage interlock and the high-voltage end of the separation system was put in a laboratory-made Plexiglass box for safety. A 4.0-mW He–Ne laser with 543.6-nm output from Uniphase (Mantence, CA, USA) was used for excitation. The light was collected with a 20× objective (numerical aperture= 0.25). One RG 610 cut-off filter was used to block scattered light before the emitted light reached the photomultiplier tube (R928, Hamamatsu, Shizuoka-Ken, Japan). The amplified currents were transferred directly through a 10-kΩ resistor to a 24-bit A/D interface at 10 Hz (Borwin, JMBS Developments, Le Fontanil, France) and stored in a personal computer.

2.2. Chemicals

The wire (75 μm in diameter) used to fabricate microchannels was obtained from Omega Engineering (Stanford, CT, USA). All chemicals used for preparing buffer solutions, PEO (M_r=8 000 000), and PVP (M_r=1 300 000) were purchased from Aldrich (Milwaukee, WI, USA). EtBr was obtained from Molecular Probes (Eugene, OR, USA). Glycine solution (100 m*M*) was adjusted with sodium tetraborate decahydrate to pH 9.1. Glycine–citrate solution (100 m*M*), pH 9.2, was prepared by adding 2.94 g trisodium citrate to 90 ml of the above glycine buffer. After citrate was dissolved completely, more glycine buffer was added to make the final volume of 100 ml. The 13-nm GNPs were synthesized according to the protocol in Ref. [32]. The concentration of thus-made GNPs is denoted by 1× (about 1.59×10^{-8} *M*) and the size of the GNP is 12.5±1.2 nm. Three aliquots of 30 ml of thus-prepared 13-nm

GNPs (weakly acidic solution containing less than 0.1 m*M* citric acid) were separately added to 50 ml 200 m*M* glycine, to 50 ml 200 m*M* glycine containing 2 *M* urea, and to 50 ml 200 m*M* glycine–citrate. Then, deionized water was added to the three solutions to bring the final volume to 100 ml. The final pH values for the three solutions were 9.1, 9.1, and 9.2, respectively. The concentrations of the GNPs are 0.3× in the solutions. To the above-prepared solutions (with/without GNPs) in beakers stirring in a water bath at 85 to 90 °C were separately and gradually added 1.5 g of PEO. After additions were completed, the suspensions were stirred for at least 1 h more. Finally, polymer solutions were degassed with a vacuum system in an ultrasonic tank. The viscosity values of the prepared 1.5% PEO solutions were between 1677 and 1682 cP. ΦX174 RF DNA-*Hae*III digest was purchased from Pharmacia Biotech (Uppsala, Sweden). DNA markers V (pBR 322/*Hae*III digest) and VI (pBR 328/*Bg1*I digest and pBR 328/*Hin*fI digest) were purchased from Boehringer Mannheim (Germany). Equal volumes of DNA markers V and VI were mixed and used in this study. The blood DNA isolation kit was obtained from Maxim Biotech (San Francisco, CA, USA). DyNAzyme II DNA polymerase and deoxyribose nucleotide triphosphates (dNTPs) were supplied from Finnzymes (Espoo, Finland). *Hpy*CH4 IV restriction enzyme was purchased from New England Biolabs (Beverly, MA, USA).

2.3. DNA extraction and PCR products

Total genomic DNA was isolated from the blood cells using QIAamp blood DNA isolation kit from QIAGEN (Hilden, Germany). The primers used to amplify the variant UGT1A7 gene were a gift from Professor Huang (unpublished result). The polymerase chain reaction mixture (100 μl) contained 1 μg DNA in 10 m*M* Tris–HCl at pH 8.8, 1.5 m*M* $MgCl_2$, 50 m*M* KCl, 0.1% Triton X-100, 200 μ*M* of each dNTP, 100 ng of each primer, and 2 U of DyNAzyme II DNA polymerase. The 35-cycle PCR reaction was performed with a Perkin-Elmer DNA thermal cycler (Norwalk, CT, USA) as follows: denaturation at 94 °C for 60 s; annealing at 55 °C for 60 s; primer extension at 72 °C for 60 s; and a final extension step at 72 °C for 10 min. The PCR product was digested with *Hpy*CH4 IV restriction enzyme prepared in a buffer composed of 50 m*M* potassium acetate, 20 m*M* Tris–acetate, 10 m*M* magnesium acetate, and 1 m*M* dithiothreitol, pH 7.9, at 37 °C for 30 min prior to CE analysis.

2.4. Microfabrication

A wire imprinting method was applied to fabricate the separation microchannels [33]. Firstly, a 75-μm wire and a PMMA plastic plate clamped between two clean glass slides was placed in the oven at 110 °C for 10 min. Then, the assembly sat at room temperature in the course of forming the microchannel. Prior to sealing, two holes of 3 mm in diameter used as buffer reservoirs and a hole of 0.8 mm in diameter for injection were drilled. Two pieces of PMMA plastics were sealed in the oven at 110 °C for 8 min. The width, depth, total length and effective length of the fabricated channel are 75 μm, 75 μm, 5 cm, and 3 cm, respectively.

2.5. Coating

The microchannels were cleaned with fresh water for 10 min and equilibrated with 5.0% PVP overnight. Then, PVP was washed out with water and the microchannels were filled and equilibrated with PEO or 0.3× 13-nm GNPs (prepared in 100 m*M* glycine, pH 9.1) at room temperature for 10 min. The microchannels coated with PVP and PEO were subjected to further coating with 0.3× 13-nm GNPs for 10 min. Prior to use, the polymer or the GNPs was flushed out and the microchannel was filled with fresh PEO or PEO containing the GNPs by pressure injection. Hereafter, PEO(GNPs) represents the PEO solution containing the 13-nm GNPs.

2.6. Separation

The DNA samples at the concentrations ranging from 10 to 25 μg/ml were conducted by dipping with a 30-cm×50 μm I.D. capillary at 10-cm height for 5 s. Although elegant electrophoretic injection providing excellent reproducibility has been used in this lab, the dipping method allows us to minimize the use of the DNA samples and provides reasonable

reproducibility [RSD of the peak height for the 603 base pairs (bp) fragment was less than 2.6% in five consecutive runs]. After injection, the injection point was covered with a piece of tape (Scotch type 810, 3M) in order to prevent the formation of bubbles. DNA migrated against a very small EOF ($<10^{-5}$ cm^2 V^{-1} s^{-1}) toward the anode when the voltage of −800 or −400 V was applied. After each run, PEO solution or PEO(GNPs) was pushed out by pressure means and the microchannel was refilled with fresh PEO or PEO(GNPs) solution.

3. Results and discussion

3.1. Dynamic coating

The microchannels fabricated in PMMA plates were coated with PVP (one layer), PVP and PEO (two layers), and PVP, PEO, and GNPs (three layers) in this study. Fig. 1A shows a poor separation of ΦX174 RF DNA-*Hae*III digests using a one-layer-coated PMMA microdevice filled with 1.5% PEO, pH 9.1. The one-layer PMMA microdevice also suffers from poor reproducibility (RSD for the migration time of the 1353 bp is 21%). Since we have demonstrated that 1.5% PEO prepared in Tris–borate buffer (pH 9.0) provides good resolution and reproducibility for the DNA fragments in CE [34], we suspected that poor reproducibility and resolution are mainly due to adsorption of the DNA fragments on PMMA. In other words, coating of PMMA with PVP is not effective. To prove our reasoning, we conducted the separation of DNA using a two-layer-coated PMMA microdevice filled with 1.5% PEO. As expected, Fig. 1B shows improved resolution and a faster separation, supporting that PVP coating was not effective to suppress EOF and to prevent the interaction with DNA on the PMMA surface. A similar result has been shown in the separation of proteins by CE using a capillary coated with two layers of polymers [35]. However, the two-layer PMMA microdevice still suffers from poor resolution and reproducibility (RSD for the migration time of the 1353 bp is 16%). Fig. 1C shows that the peak profiles were sharper and the resolution improved when using a three-layer-coated PMMA microdevice filled with 1.5% PEO. Still, poor reproducibility

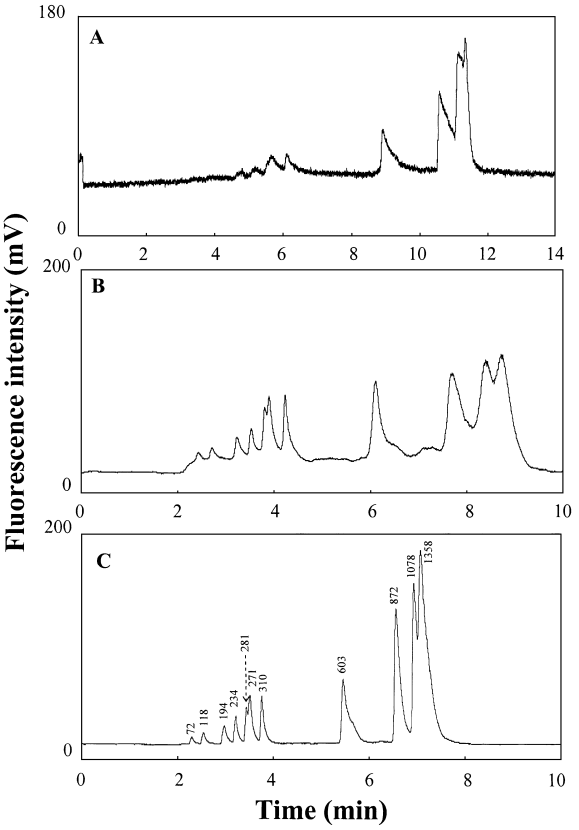

Fig. 1. Comparison of the separations of 25 μg/ml ΦX174 RF DNA-*Hae*III digest using one-, two-, and three-layer-coated PMMA microdevices. One-layer coating: 5.0% PVP overnight in (A); two-layer coating: 5.0% PVP overnight and then 1.5% PEO for 10 min in (B); three-layer coating: 5.0% PVP overnight, 1.5% PEO for 10 min, and 0.3× 13-nm GNPs for 10 min in (C). The width, depth, total length, and effective length of the microchannel were 75 μm, 75 μm, 5 and 3 cm, respectively. PEO (1.5%) was prepared in 100 m*M* glycine buffer containing 0.5 μg/ml EtBr, pH 9.1. Hydrodynamic injections were conducted by dipping the DNA sample with a 30 cm×50 μm I.D. capillary. The separations were conducted at −800 V.

(RSD for the migration time of the 1353 bp is 13%) is problematic, which is likely due to desorption of the GNPs or the interaction between DNA and the GNPs [29]. Because the currents changed slightly in the three cases (15–17 μA), a faster separation shown in Fig. 1C is not due to decreases in viscosity as a result of Joule heating, but due to a small EOF. We note that the PVP- and PEO-modified GNPs are neutral and stable due to steric effects (an unpublished result). To this end, we conclude that the

three-layer-coated PMMA microdevice provides the best performance and is possibly suitable for further studies.

3.2. Effect of background electrolytes

To support the role of the GNPs in improving resolution and reproducibility, we first tested the effect of ionic strength using the three-layer-coated PMMA microdevice. The adsorption of macromolecules is suppressed at high ionic strengths, leading to better reproducibility and greater efficiency. Ionic strength also plays an important role in determining the electrophoretic mobility of macromolecules. In this study, 1.5% PEO solutions separately prepared in 50, 100, and 200 m*M* glycine buffers, pH 9.1, were tested. The electrophoretic mobilities of the DNA fragments decreased with increasing glycine concentration (50–100) as expected, while slightly changed in the range of 100–200 m*M* glycine due also partially to Joule heats (currents were 17 and 31 μA in 100 and 200 m*M* glycine, respectively). The fact that the reproducibility slightly worsened with increasing glycine concentration as shown in Table 1 rules out the possibility of optimizing resolution and reproducibility by controlling the ionic strength of 1.5% PEO. Poor reproducibility is probably due to desorption of the GNPs from the separation channel wall at high glycine concentrations. One other drawback of using 1.5% PEO as a sieving matrix is a need to coat the microchannel wall with 1.5% PEO and the GNPs after each run, which is problematic with respect to high throughput. These disadvantages were circumvented by conducting the separation using 1.5% PEO(GNPs) as also shown in Table 1. Please note that the reproducibility is reasonable (RSD less than 2.5%), supporting our reasoning that poor reproducibility is due to desorption of GNPs when using 1.5% PEO only. It is noted that the separation was complete in 8 min, which is comparable to those obtained by microchip electrophoresis using cellulose derivatives [26,36].

Fig. 2A shows the separation of DNA markers V and VI (18–2176 bp) using 1.5% PEO(GNPs), with an unsatisfactory result when compared to that shown in CE [14]. To further improve resolution, we added urea to 1.5% PEO(GNPs). Fig. 2B shows slightly improved resolution, but at the expense of a longer separation time, when the separation was carried out using 1.5% PEO(GNPs) in the presence of 1 *M* urea. Please note that the viscosity (1680 cP) of 1.5% PEO(GNPs) did not increase in the presence of 1 *M* urea [37]. Thus a longer separation time is likely due to slight changes in DNA conformation and/or the change in the morphology of PEO matrices. With further increases in urea concentration, the loss of resolution was found, mainly due to denaturation of DNA [38,39]. In a previous study, we have shown that the GNPs aggregate slightly in the presence of PEO at the concentration higher than its entanglement threshold concentration (0.07%). To minimize the aggregation, we used 100 m*M* glycine–citrate buffer, pH 9.2, to prepare 1.5% PEO(GNPs). Fig. 2C shows that using the 1.5% PEO(GNPs) the

Table 1
Effect of the buffers used to prepare 1.5% PEO on the electrophoretic mobility of DNA

DNA fragment (bp)	Electrophoretic mobility (10^{-4} cm^2 s^{-1} V^{-1}) (RSD%, n=5)			
	50 m*M* Glycine	100 m*M* Glycine	200 m*M* Glycine	100 m*M* Glycine+0.3× GNPs
72	1.69 (9.8)	1.36 (7.1)	1.37 (9.8)	1.38 (1.1)
118	1.48 (8.7)	1.23 (9.8)	1.24 (8.5)	1.24 (1.4)
194	1.21 (7.8)	1.05 (8.3)	1.06 (8.4)	0.99 (1.5)
234	1.11 (7.5)	0.97 (8.8)	0.97 (7.6)	0.97 (1.3)
281	1.04 (5.8)	0.96 (7.4)	0.91 (8.1)	0.89 (1.5)
271	1.01 (5.4)	0.89 (7.7)	0.89 (9.7)	0.88 (1.9)
310	0.93 (12)	0.88 (10)	0.88 (12)	0.83 (2.3)
603	0.63 (7.9)	0.57 (8.3)	0.46 (9.5)	0.59 (2.5)
872	0.54 (10)	0.47 (13)	0.39 (11)	0.48 (2.1)
1078	0.51 (9.6)	0.45 (11)	0.31 (13)	0.45 (2.3)
1353	0.45 (8.7)	0.44 (12)	0.30 (14)	0.44 (2.4)

Conditions were as in Fig. 1C.

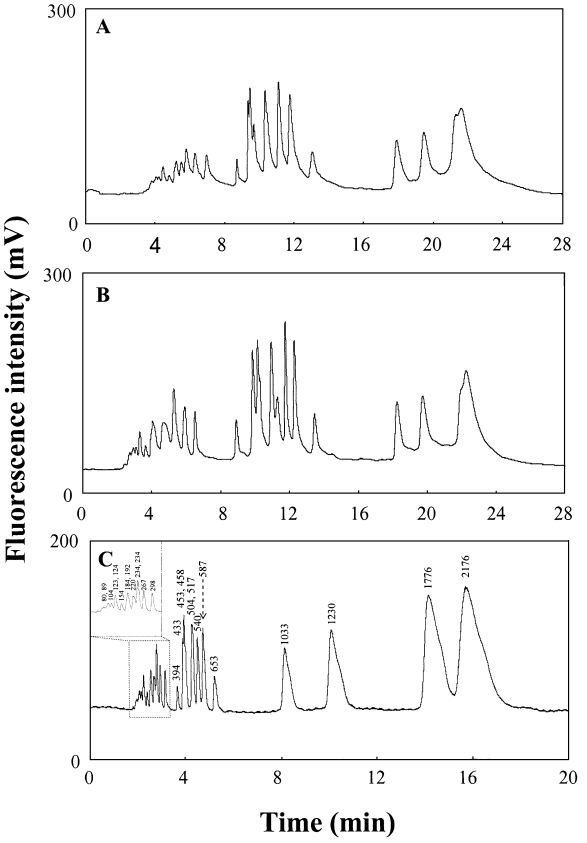

Fig. 2. Separations of a mixture of equal volume of 10 μg/ml DNA markers V and VI under different conditions using a three-layer-coated PMMA microdevice. PEO(GNPs) (1.5%) solutions containing 0.5 μg/ml EtBr were prepared in 100 m*M* glycine buffer, pH 9.1 in (A); 100 m*M* glycine buffer, pH 9.1, containing 1 *M* urea in (B); and 100 m*M* glycine–citrate buffer, pH 9.2 in (C). Current: 20 μA in (A) and (B), 30 μA in (C). The separations were conducted at −400 V. Other conditions were as in Fig. 1.

separation was much faster (18 min) and the resolution for the small DNA fragments improved when compared to those shown in Fig. 2A and B. The increases in the electrophoretic mobility of DNA are partially due to the interactions between citrate ions (triprotic acid) and the DNA fragments, which is supported by increasing the electrophoretic mobility of DNA in 1.5% PEO with increasing citrate concentration (not shown). Please note that the separation was unsuccessful (poor resolution) in the absence of the GNPs. A faster separation time is also slightly due to Joule heating (30 μA vs. 18 μA).

When comparing the three electropherograms, it is obvious that the separation time and resolution are quite different. In addition to the reasoning addressed above, the changes in the morphology of the PEO matrices should also be a contributor. It is supported by different colors of the PEO(GNPs) solutions; the PEO(GNPs) in the presence of a small amount of citrate or 1 *M* urea is dark blue (red shift), while it is ruby red (due to surface plasmon absorption of the GNPs with a maximum absorption wavelength at 520 nm) in the presence of 100 m*M* citrate. The red shift is due to aggregation of the GNPs, with the support of the transmission electron microscopy (TEM) images as shown in Fig. 3. It has been reported that citrate is a good capping agent to protect the GNPs [32]. Although aggregation occurred, the PEO(GNPs) solutions were all stable for more than 1 month, supported by no change in the UV–Vis absorption spectra and only a very small amount of GNPs precipitated after centrifugation at 18 000 rev./min for 15 min. In the end, we conclude that the separation results shown in Fig. 2 are related to different morphologies of the three PEO(GNPs) that possibly led to different interactions of DNA with PEO and GNPs.

3.3. Stepwise changes in EtBr

We have shown that a technique based on stepwise changes in EtBr during separation allows optimized resolution, speed and sensitivity [40]. This is mainly due to the fact that the electrophoretic mobility, fluorescence intensity, and structure of the DNA–EtBr complexes are sensitive to the concentration ratio of EtBr/DNA. Once DNA intercalates with EtBr, the electrophoretic mobility decreases, the fluorescence intensity increases, and the structure becomes more rigid. Thus it is possible to further improve resolution for the small DNA fragments (<653 bp) when conducting the separation at high EtBr concentrations. However, long migration times for the large DNA fragments are problematic using such conditions. To prevent this problem, we conducted the separation using 1.5% PEO(GNPs) containing 5 and 0.5 μg/ml EtBr in the separation channel and the anodic vial, respectively (Fig. 4). Since DNA (toward anode) and EtBr (toward cathode) migrated in the opposite direction, the DNA

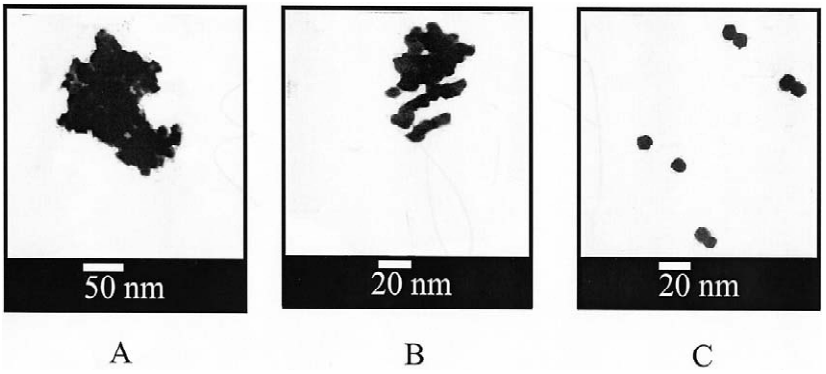

Fig. 3. TEM images of 1.5% PEO(GNPs) prepared in three different buffers shown in Fig. 2. Conditions for A, B, and C correspond to those shown in Fig. 2A–C, respectively.

fragments interacted with different concentrations of EtBr in the course of the separation. The small DNA fragments take the most time to intercalate with EtBr at high concentrations, leading to longer migration times, stronger fluorescence intensity, and better resolution when compared to Fig. 2C. Under such high EtBr concentrations, it also allows the detection of the two small DNA fragments (18 and 21 bp). On the other hand, the large DNA fragments did not take the most time to intercalate with EtBr at low concentrations, with the support of a long separation time when compared to that shown in Fig. 2C. Please note that the separation under static conditions (5 μg/ml EtBr) was much longer (45 min) and a loss of resolution for the large DNA fragments was found. Although the separation time is long (28 min), this is

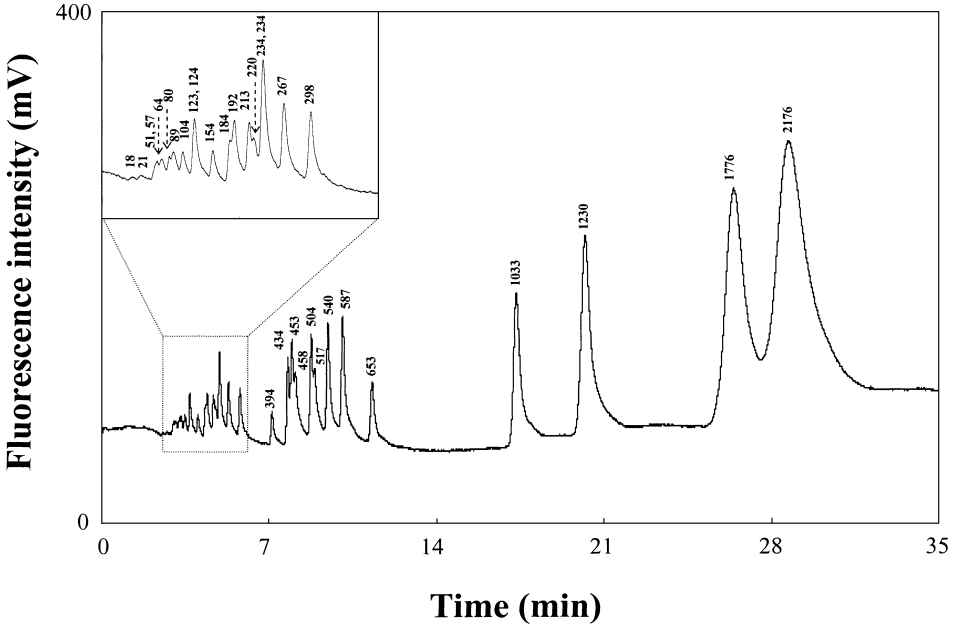

Fig. 4. Electropherogram of the DNA separation by a stepwise microchip CE technique. The concentrations of EtBr were 5 and 0.5 μg/ml in the separation channel and the anodic reservoir, respectively. Other conditions were as in Fig. 2C.

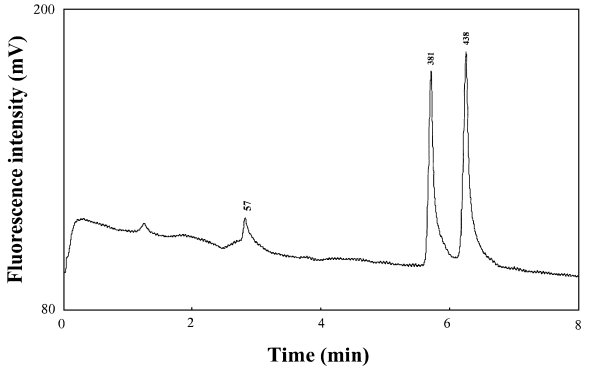

Fig. 5. Separation of the digested PCR products (UGT1A7 gene −57 T→G). Other conditions were as in Fig. 2C.

the first example showing the separation of such a wide range of DNA fragments (18–2176 bp) in microchip electrophoresis.

3.4. Analysis of PCR products

The analysis of PCR products is of considerable importance in many fields such as the medical realm and forensics [41,42]. The polymorphisms of UGT1A7 gene are related to hepatocellular carcinoma and the separation of one of their PCR products was tested using the three-layer-coated PMMA microdevice filled with 1.5% PEO (GNPs) (pH 9.2) [43]. When the 438-bp PCR product of a mutant DNA (UGT1A7 gene −57 T→G) is digested with a restriction enzyme (*Hpy*CH4 IV), two fragments of size 57 and 381 bp were formed, respectively. The electropherogram shown in Fig. 5 presents three peaks corresponding to the 57-, 381-, and 438-bp fragments, indicating that the DNA sample is mutant. Table 2 shows the reproducibility of this method, with RSD values of the migration time and the peak height for the three peaks less than 2.0 and 2.3%, respectively, in five consecutive runs. The chip-to-chip RSD values for the three peaks were also less than 4.9 and 5.4% in five different chips, showing the potential of this simple and robust method for single nucleotide polymorphisms (SNPs).

4. Conclusions

We have demonstrated a simple approach to coating the wall of the separation channels fabricated on PMMA plates using PVP, PEO, and GNPs in sequence. The three-layer-coated PMMA microdevice provides improvements in resolution and reproducibility for DNA separation when using 1.5% PEO(GNPs), allowing the separation of DNA fragments ranging in size from 18 to 2176 bp. In this study, we have also found that citrate stabilizes the GNPs in 1.5% PEO as well as affecting the electrophoretic mobility of DNA, and thus its concentration is an important parameter for determining resolution and speed. Although the three-layer-coated PMMA microdevice is useful and cost-effective, the speed and efficiency demonstrated in this study is not quite impressive for high-throughput DNA analysis (e.g. SNPs of UGT1A7 gene), mainly because of wider separation channels (75 μm) and large amounts of injected samples. To speed up DNA separation, small separation channels (e.g. 20 μm) that provide great efficiency and allow faster separation at high electric fields are needed. To avoid conducting the separation at high ionic strengths, a low conductivity medium might be used instead of 100 m*M* glycine–citrate. Very recently, we have found GNPs are stable in 10 m*M* glycine containing small amounts of thiols such as 3-mercaptopropionic acid (a good capping agent for GNPs). To improve efficiency and reproducibility, electrophoretic injections of small

Table 2
Migration time and peak height for the analysis of the digested PCR products of UGT1A7 gene

DNA fragment (bp)	Avg. migration time (min) (RSD%, $n=5$)		Avg. peak height (mV) (RSD%, $n=5$)	
	Run-to-run	Chip-to-chip	Run-to-run	Chip-to-chip
57	2.9 (1.1)	3.1 (4.5)	10.8 (1.9)	9.9 (4.8)
381	5.7 (2.0)	5.5 (4.9)	75.3 (2.3)	77.8 (5.3)
438	6.4 (1.2)	6.1 (4.9)	84.5 (2.3)	88.7 (5.4)

Conditions were as in Fig. 2C.

amounts of DNA samples have to be conducted when using a small separation channel.

Acknowledgements

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