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PAPER

Disposable polyester–toner electrophoresis microchips for DNA analysis

Gabriela R. M. Duarte,[abc] Wendell K. T. Coltro,[bd] Juliane C. Borba,[ab] Carol W. Price,[e] James P. Landers[efg] and Emanuel Carrilho[*ab]

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Microchip electrophoresis has become a powerful tool for DNA separation, offering all of the advantages typically associated with miniaturized techniques: high speed, high resolution, ease of automation, and great versatility for both routine and research applications. Various substrate materials have been used to produce microchips for DNA separations, including conventional (glass, silicon, and quartz) and alternative (polymers) platforms. In this study, we perform DNA separation in a simple and low-cost polyester–toner (PeT)-based electrophoresis microchip. PeT devices were fabricated by a direct-printing process using a 600 dpi-resolution laser printer. DNA separations were performed on PeT chip with channels filled with polymer solutions (0.5% m/v hydroxyethylcellulose or hydroxypropylcellulose) at electric fields ranging from 100 to 300 V cm^{-1}. Separation of DNA fragments between 100 and 1000 bp, with good correlation of the size of DNA fragments and mobility, was achieved in this system. Although the mobility increased with increasing electric field, separations showed the same profile regardless of the electric field. The system provided good separation efficiency (215 000 plates per m for the 500 bp fragment) and the separation was completed in 4 min for 1000 bp fragment ladder. The cost of a given chip is approximately $0.15 and it takes less than 10 minutes to prepare a single device.

Introduction

Toner and paper-based devices stand out as two promising platforms for microfluidic applications at very low cost. Both substrate materials are inexpensive and the fabrication process only requires readily accessible, non-scientific instrumentation with fabrication that is time-efficient and does not require cleanroom facilities.[1] Polyester–toner (PeT) electrophoresis devices have exhibited a great potential for bioanalytical analysis.[2] PeT chips can be fabricated in a matter of minutes using a direct-printing process, which makes possible the production of tens of devices on a single transparency sheet (letter/A4 size) with consumables that cost less than 1.0 USD. The microfluidic architecture is defined by the white regions of a drawing, which is interpreted by a laser printer as an instruction to avoid the deposition of toner particles. The sealing of the microfluidic channels is provided quickly by a lamination step on a hot press.

PeT electrophoresis devices have been integrated with electrochemical[3–9] and fluorescence detectors[9] to monitor the separation of inorganic species, neurotransmitters, as well as pharmaceutical compounds. In close comparison to the most popular microfluidic platforms, like glass and PDMS, PeT devices exhibit the lowest electroosmotic flow (EOF) velocity.[10] This characteristic can be useful for separations where EOF needs to be suppressed while leaving the channel surface hydrophilic in nature – as such, surface pretreatment is circumvented. While PeT chips are seeing an increased adoption for simple microfluidic chip-based applications,[10] there is no report in the literature describing the use of PeT chips for DNA separations. This is significant for a number of reasons. First, DNA separations have been traditionally carried out on glass chips, but there is a major shift towards polymeric devices due to a cost-based driving force. PeT chips certainly fall into that regime. Seeded by the key chip-based DNA separation developments from Mathies' group who first reported the separation of DNA fragments ranging from 70 to 1000 bp in 120 seconds,[11] separation technology has advanced substantially. Multiple groups have contributed to the evolution of this,[12–14] showing the ease with which high-resolution DNA separations could be achieved. This has led to a focus on the integration of all analytical steps involved in genetic analysis (extraction, amplification, and separation) onto the same chip.[15] In this respect, PeT microchips have begun to show the same capabilities. They show the ability

[a]*Instituto de Química de São Carlos, Universidade de São Paulo, Grupo de Bioanalítica, Microfabricação e Separações, Avenida Trabalhador São-carlense 400, P.O. Box 780, 13566-590 São Carlos, SP, Brazil. E-mail: emanuel@iqsc.usp.br; Tel: +55 (16) 3373-9441*
[b]*Instituto Nacional de Ciência e Tecnologia de Bioanalítica, 13083-970 Campinas, SP, Brazil*
[c]*Universidade Estadual de Goiás, 75132-903 Anápolis, GO, Brazil*
[d]*Instituto de Química, Universidade Federal de Goiás, 74001-970 Goiânia, GO, Brazil*
[e]*Department of Chemistry, University of Virginia, Charlottesville, Virginia 22904, USA*
[f]*Department of Mechanical Engineering, University of Virginia, Charlottesville, Virginia 22904, USA*
[g]*Department of Pathology, University of Virginia Health Science Center, Charlottesville, Virginia 22908, USA*

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to combine the efficient dynamic solid phase extraction (dSPE) of DNA from whole blood with a defined compatibility with downstream microchip-based PCR amplification.[2] With SPE and PCR carried out on PeT devices,[2] the remaining challenge is to demonstrate that this can be seamlessly interfaced with DNA separation, the linchpin to a fully integrated, disposable microdevice for genetic analysis.

Coltro *et al.*[10] compared the analytical performance of electrophoresis PeT microchips against glass and PDMS devices. They found that PeT exhibited the lowest EOF of all devices tested, an attribute that was useful for applications that demand low- or no-EOF conditions. While the inherent low EOF is clearly advantageous for separations like DNA, and circumvents the need for coating the channels, they stated that PeT microchips presented a major drawback over the other chip substrates – low separation efficiency/poor resolution and the length of injection plug. In the same work, Coltro and co-workers demonstrated that the contribution of the channel wall, σ^2_{wall}, accounted for almost 90% of the total variance, where in the glass chip, this parameter was <30%. Since the geometries of all devices compared were essentially identical, the poor performance of the PeT chips was attributed to the roughness of the channel wall. If so, low separation efficiencies pose a challenge for the separation of DNA fragments.

Here we show that, indeed, DNA separation on PeT chips can be accomplished with reasonable resolution and separation efficiency. Focusing in on key analytical parameters that affect the separation efficiency, *e.g.*, the effect of electric field strength on the electrophoretic mobility, run-to-run reproducibility, we demonstrate that PeT chips provide (i) good sizing linearity for DNA ladders, (ii) good sizing accuracy and adequate precision for PCR amplicons, (iii) compatibility with different polymer solutions, and (iv) good agreement with known mechanisms for DNA migration. Only then can we discuss the analytical feasibility of using PeT chips to perform DNA separations, integrated with sample preparation steps (SPE and PCR), as already reported with glass microchip platforms.[15]

Materials and methods

Reagents

Ethylenediaminetetraacetic acid tetrasodium salt (EDTA) was purchased from Sigma Chemical (St Louis, MO, USA); tris(hydroxymethyl)aminomethane (Tris) was from Mallinckrodt (St Louis, MO, USA); DNA ladder (100 bp) was either from Ludwig Biotec (Porto Alegre, RS, Brazil) or from New England BioLabs (Beverly, MA, USA); hydroxyethylcellulose (HEC, 90 000–105 000 M_w) was from Fluka (Buchs, Switzerland) and hydroxypropylcellulose (HPC, 100 000 M_w) was from Acros Organics (Fisher Scientific, Pittsburgh, PA); PCR reagents were obtained from Fisher Scientific (Fairlawn, NJ, USA). Primers for amplification of a 520 bp fragment of λ-phage were synthesized by MWG Biotech (High Point, NC, USA). PicoGreen® intercalating dye was obtained from Invitrogen (Carlsbad, CA, USA). Transparency films (CG 3300 model) and toner cartridge (Q2612A) were obtained from 3M (São Paulo, SP, Brazil) and Hewlett Packard (Palo Alto, CA, USA) respectively.

Fabrication of microdevices

PeT microchips were fabricated by a direct-printing process with a double toner layer structure according to the procedure described elsewhere.[3,4] Fig. 1A exhibits the basic steps of the microfabrication process based on direct-printing technology.[3,4] Briefly, the layout of the device and its mirror image were printed over the same polyester sheet using a 600 dpi-resolution laser printer. In order to have access to microfluidic channels, access holes were created on the mirrored layout using a paper punch. Following this procedure, the layout and its perforated mirror image were aligned and laminated using a standard office laminator at 120 °C at a rate of 30 cm min^{-1}. This lamination step accomplished the sealing of both PeT films in a few seconds. After the sealing step, the bases of 100 μL pipette tips were glued with epoxy resin over the holes to form solution reservoirs. The microchannels were produced in a simple cross-format (see Fig. 1B) with 200 μm width and 12 μm height. The injection and separation channels were 10 and 40 mm long, respectively. The plug length was 620 μm and the volume injected was 1.5 nL.

Preparation of devices

The channel was filled by capillary action with either a 0.5% HEC or 0.5% HPC solution. Typically, an aliquot of 25 μL of polymer solution was added to the buffer reservoir and all channels were completely filled in *ca.* 2 min. Afterwards, 25 μL of 0.1× TE (1 mM Tris/0.1 mM EDTA, pH 8.0) was added to the same reservoir. The buffer waste reservoir was also filled with a volume of 50 μL (25 μL of 0.5% HEC/HPC and 25 μL of 0.1× TE solutions). A volume of 50 μL of 0.1× TE solution was added to both sample and sample waste reservoirs. To check if the channels were completely filled and that there were no bubbles in the channels, an electric field of 200 V cm^{-1} was applied to both channels. A stable current indicated that the channel was uniformly filled with the polymer solution, *i.e.*, that there were no bubbles in the channels. After conditioning, the buffer in the

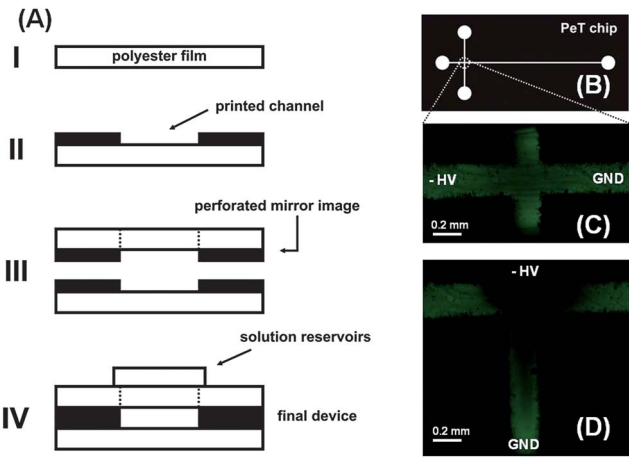

Fig. 1 Representation of the (A) microfabrication process and (B) design of PeT electrophoresis devices for DNA separation. Images showing (C) the electrokinetic loading of the sample and (D) plug injection inside the separation channel of a PeT device. In (A), step IV, solution reservoirs are created by gluing the bases of 100 μL pipette tips with epoxy resin. (A) and (B) are not to scale.

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sample reservoir was replaced by 50 μL of PicoGreen®-labeled sample and electrophoresis separation was performed.

Electrophoresis procedures and instrumentation

The electrokinetic transport of the flow into microfluidic channels was accomplished by a bipolar single-channel high-voltage power supply (CZE 1000R, Spellman, Hauppauge, NY, USA) controlled by a computer equipped with a National Instruments (NI) interface (USB-6009 model). Electrokinetic injections were performed using an unpinched injection procedure (Fig. 1C and D). For the present experiments, the injections were performed by applying a desired potential of 100 V cm^{-1} for 30 s to the sample reservoir with the sample waste reservoir grounded, and all other reservoirs floating. Switching the high-voltage contacts and applying the corresponding separation voltages to the running buffer reservoir, while maintaining the detection reservoir grounded, and all other reservoirs floating, performed the separations. Fig. 1C and D show fluorescence images of the loading of the injection channel with sample and the introduction of a sample plug inside the separation channel, respectively. Based on the fluorescence image depicted in Fig. 1C, the injection volume has been estimated to be *ca.* 1.5 nL.

Laser-induced fluorescence detection was performed employing a compact system (IS Biotech, Porto Alegre, RS, Brazil) equipped with a 488 nm argon ion laser beam with optical output power adjustable from 1 to 50 mW (LaserPhysics, Salt Lake City, UT, USA). Detection was performed at 34 mm from the injection point. The resulting fluorescence signal was sent to the NI interface and monitored in real time using a program written in *LabVIEW*®.

Sample preparation

The DNA ladder was intercalated with PicoGreen® by mixing 12.5 μL of a DNA (100 ng μL^{-1}) with 12.5 μL of 0.1× TE and 25 μL of 0.5% PicoGreen® in 0.1× TE. The mixture was then incubated for 10 min in the dark. PCR product was intercalated with PicoGreen® by mixing 2 μL PCR product with 18 μL of 0.1× TE and 25 μL of 0.5% PicoGreen® in 0.1× TE followed by incubation for 10 min in the dark.

DNA amplification

PCR amplification of a portion of λ-phage DNA was performed on a Bio-Rad MyCycler (Hercules, CA, USA) using primers to specifically amplify a 520 bp DNA sequence. The PCR reaction contained 10 mM Tris–HCl buffer, pH 8.3, 50 mM KCl, 82.4 mM $MgCl_2$, 0.4 mM of each primer, 0.2 mM of each dNTP, 1 ng μL^{-1} λ-phage DNA, 0.24 mg mL^{-1} of bovine serum albumin (BSA), 0.1 units per μL Taq polymerase. The thermocycling conditions were as follows: 120 s at 95 °C for initial DNA denaturation, 30 cycles of 30 s each at 95 °C (denaturation) and 68 °C (annealing/extension), followed by 120 s at 72 °C for a final extension. The PCR product was divided and analyzed by the PeT microchip and Bioanalyzer 2100 (Agilent Technologies, Palo Alto, CA) according to the manufacturer's instructions.

Results and discussion

Of the many advantages that PeT microchips possess over the other chip substrates, most notable are ease of fabrication, simple instrumental requirements and low cost. Moreover, when compared to conventional microfluidic platforms, *e.g.*, glass and PDMS, PeT devices exhibit the lowest EOF magnitude (0.35×10^{-4} cm^2 V^{-1} s^{-1}) and separation efficiency. Table 1 highlights some of the features of PeT microchips in comparison with other substrates and a commercial DNA analyzer based on microchip separation (Bioanalyzer).

Quantitative studies have demonstrated that the toner wall contributes *ca.* 90% to the band broadening. This result can be attributed to the analyte–wall interaction as well as to the roughness of the channel walls.[10] Despite these drawbacks, PeT chips have a good potential for implementation with a number of applications, including genetic analysis, where the low EOF magnitude can be beneficial. This is particularly so for sieving-based DNA separations, where a low EOF can ensure the stability of the separation matrix in the microchannels over an extended time, *i.e.*, during successive injections. This is an attractive feature for integrated analysis where sequential repeated injections are done to ensure peak identification.[15] DNA analyses have been extensively explored on glass microfluidic platforms.[16,17] In this respect, one disadvantage of glass is its high EOF, which needs to be suppressed to avoid the electrokinetic transport of the sieving matrix (polymer network) solution. One of the purposes of this communication is to investigate the run-to-run repeatability of DNA fragment separation on PeT devices with low EOF magnitude.

DNA separation of 100 bp DNA ladder on PeT microchips

The literature describes DNA separations in microdevices carried out at voltages that range from 70–300 V cm^{-1}. For this reason we attempted DNA separations under electric fields that ranged from 75 to 300 V cm^{-1}. Fig. 2A shows an

Table 1 Comparison of PeT microchip with glass chip and other microchips

	PeT chip	Glass chip	Native PDMS chip	Bioanalyzer
Cost per chip	~$0.15	~$40.0	~$2 to 5	~$40.0
Time to fabricate	Less than 10 min	~24 hours	~3 to 4 hours	—
EOF ($\times 10^{-4}$ cm^2 V^{-1} s^{-1})[a]	0.35	3.90	1.12	The same value as for glass chip (3.90)
Durability	Disposable, but can be reused a few times	Reusable	Reusable, a few times	Single use, with 12 runs per chip

[a] EOF values were extracted from ref. 10.

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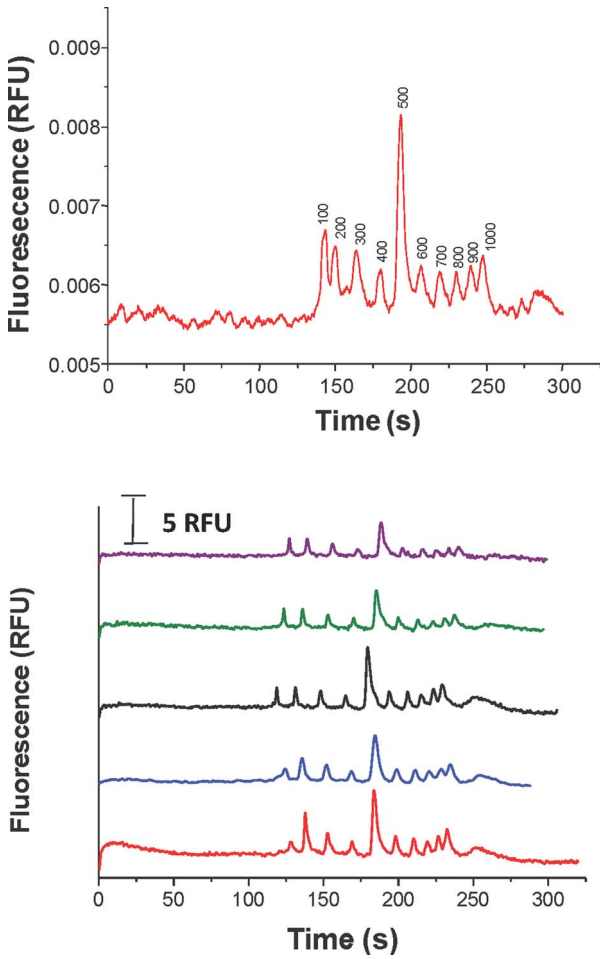

Fig. 2 (A) Electropherogram from a separation of 100 bp DNA ladder under electric field of 200 V cm^{-1}. DNA concentration: 25 ng μL^{-1}; electrokinetic injection: 300 V per 30 s; electric field of separation: 200 V cm^{-1}; confocal laser-induced fluorescence detection system equipped with a 488 nm argon ion laser for excitation. Effective separation length was 34 mm from the injection point. (B) Five consecutive injections of DNA ladder with 100–1000 bp fragments on the same microchip and sieving matrix (0.5% HEC). Injections order from first (bottom) to fifth (top). Experimental conditions: electric field of 200 V cm^{-1}; DNA concentration: 25 ng μL^{-1}; electrokinetic injection: 300 V per 30 s; electric field of separation: 200 V cm^{-1}; confocal laser-induced fluorescence detection system equipped with a 488 nm argon ion laser for excitation. Effective separation length was 34 mm from the injection point.

electropherogram of a 100 bp DNA ladder under application of 200 V cm^{-1} and 0.5% HEC solution.

With the application of 100 V cm^{-1} to the separation channel, the analysis time was unacceptably long in microfluidic terms (12 min) and the peaks broad. Doubling the electric field to 200 V cm^{-1} improved the electrophoretic resolution substantially (>1 for all peaks) and analysis time was reduced to *ca.* 4 min. At 300 V cm^{-1}, the resolution between peaks larger than 700 bp was <1; therefore, an electric field of 200 V cm^{-1} was deemed optimal for this sieving matrix/chip system. This electric field presented a good balance between the resolution (R_s) and total analysis time for this separation. Electrophoretic resolution is an important parameter to evaluate the performance of a separation method and to compare with other methods. Resolution is defined by the ratio of the distance between two adjacent peaks, Δt, to the average peak width measured at the base $(W_{1b} + W_{2b})/2$, and was calculated according to eqn (1):

$$R_s = 2\Delta t_m/(W_{1b} + W_{2b}) \quad (1)$$

Table 2 shows the resolution between all peaks, calculated by eqn (1), under application of 200 V cm^{-1}. The values in Table 2 show that the separation at 200 V cm^{-1} yielded resolution values (R_s) greater than 1 between each pair of peaks in the ladder. For DNA sizing, it is convenient to normalize the resolution in terms of base pair (R_{bp}), an important parameter to evaluate the capacity of the system to distinguish DNA molecules of similar size, *i.e.*, the smallest difference in size between DNA fragments that can be resolved. The resolution per base pair was calculated according to eqn (2):

$$R_{bp} = \Delta t/(\Delta N W_h) \quad (2)$$

where ΔN is the size difference between the two fragments in question and W_h the width of the peak. When using an array of closely spaced size standards (DNA ladder), the peak width does not change significantly from peak to peak, and we can use the width of a single peak (we used the first peak of the pair) instead of the average peak width.[18] For the electropherogram resulting from separation of the 100 bp ladder, the range of R_{bp} values was 37.2–91.5 (Table 2); the average of R_{bp} found for PeT microchips was 56 bp. Each DNA-based application has its specific requirement with respect to the separation, depending on the number and length of the DNA fragments to be resolved. The typical non-sequencing genomic analysis requires, on average, at least 10 bp resolution – this includes diagnostic assays that verify the PCR amplification of the correct DNA targets to avoid false positive identification and gene expression.[19]

Separation efficiency

The analytical performance, evaluated as separation efficiency (in terms of number of plates, N), was calculated according to eqn (3):

Table 2 Resolution calculated for each pair of peaks of a 100 bp DNA ladder using eqn (1) and (2), respectively, and separation efficiency calculated for each peak (in plates per m) using eqn (3). Data obtained in a PeT microchip using HPC 0.5% as matrix and 200 V cm^{-1} applied over the separation channel of 4 cm in total length

DNA size (bp)	R_s[a]	R_{bp}[a]	$N_{h/2}$ (plates per m)
100	—	—	138 000
200	1.20	67.3	73 000
300	2.06	49.9	78 000
400	2.58	37.2	176 000
500	2.73	45.1	215 000
600	2.10	43.8	95 000
700	1.68	51.9	154 300
800	1.65	58.4	178 000
900	1.59	58.7	143 600
1000	1.04	91.5	231 000
Average	1.85	55.9	148 200

[a] Resolution measured between each DNA size marker and the previous one.

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$$N = 5.55(t_m/W_{h/2})^2 \quad (3)$$

where t_m is the migration time and $W_{h/2}$ is the peak width at half-height. Table 2 shows the separation efficiencies calculated for all peaks. The separation efficiencies for the separation of DNA fragments in PeT microchips were poorer than those in glass microchips using the same sieving matrix (HEC). Tian and Landers[20] showed that, for a 504 bp DNA fragment, the separation efficiency was 683 000 plates per m in a glass chip with an effective separation length of 8.5 cm; separation efficiency in the PeT microchip for a 500 bp fragment was 215 000 plates per m, representing a 3-fold decrease compared to the separation efficiency obtained by Tian and Landers in a glass chip for fragments with similar size. The differences can be related to the quality of channels, inherent to the fabrication process of PeT microchips. Toner-based devices are created just by a single printing step using a laser printer to deposit a toner layer that defines the channel wall; after the sealing step, channels exist where the printer did not deposit toner. Hence, a 'surface roughness' is associated with the walls. In addition to toner walls, the random presence of toner particles inside microchannels is commonly observed. It is clear that this combination negatively affects the separation efficiency.[3]

In addition to the effects of the wall, not surprisingly, the injection plug length also has a significant influence on the separation efficiency. The contributions to total variance, σ_T^2, in microchip electrophoresis are:

$$\sigma_T^2 = \sigma_{diff}^2 + \sigma_{inj}^2 + \sigma_{det}^2 + \sigma_{wall}^2 + \sigma_J^2 \quad (4)$$

where σ_{diff}^2 is from the molecular diffusion, σ_{inj}^2 is from the injection system, σ_{det}^2 is from the detector system, σ_{wall}^2 is from channel wall, and σ_J^2 is from Joule heating.[10] Once the PeT shows efficient heat dissipation, the σ_J^2 term can be considered negligible compared with σ_T^2.

For a 100 bp DNA fragment the total variance (σ_T^2) was 2.5×10^5 μm^2, and the value of σ_{diff}^2 for DNA fragments on porous matrix was 68 μm^2. The contribution of the injection, σ_{inj}^2, to the total variance has been estimated according to the equation $\sigma_{inj}^2 = (l_{inj}^2/12)$, where l_{inj} means the sample zone length injected inside a microchannel. Based on the fluorescence image depicted in Fig. 1C, l_{inj} is *ca.* 620 μm, resulting in a σ_{inj}^2 value of 3.2×10^4 μm^2. Considering a σ_{det}^2 ($l_{det}^2/12$) value of 208 μm^2, the σ_{wall}^2 value was estimated to be 2.2×10^5 μm^2. These values indicate that σ_{wall}^2 was the largest contributor (*ca.* 87%) to the band broadening for the PeT microchip, confirming our concerns over physical contributions of the toner. The σ_{inj}^2 also contributes significantly with *ca.* 12.8% to the total variance. This systematic study is in agreement with data previously reported.[10] Overall, the elevated roughness of the wall and the length of injection plug contributed to the low efficiency and resolution of the PeT chip. Drawbacks acknowledged, PeT devices have been demonstrated to be capable of the electrophoretic separation of fragments between 100 and 1000 bp, and with reasonable resolution. However, improvement of the system and the experimental conditions will be needed in order to provide the resolution required for select DNA applications. Of particular importance will be channels with decreased surface roughness which might be obtainable with high resolution printers, *e.g.*, 2400 dpi and higher.

Influence of electric field on mobility

Fig. 3A shows the relationship between DNA fragment mobility (μ) and log 1/bp under varying electric field strengths. The mobility of DNA is independent of the electric field strength in the sieving regime and in the non-oriented reptation regime. With increasing electric field, the onset of reptation with orientation is shifted to smaller DNA sizes, reducing the size range to be effectively separated. The data show that the increase of electric field affected the electrophoretic mobility (for those fragments larger than 500 bp) as expected, since high electric field promotes the alignment of larger DNA molecules with electric field suppressing the reptation of DNA molecules[21] and inducing the biased reptation regime.[22] It is clear that the effect of the electric field on electrophoretic mobility is more accentuated (noticeable) for long fragments. Even though lower electric field

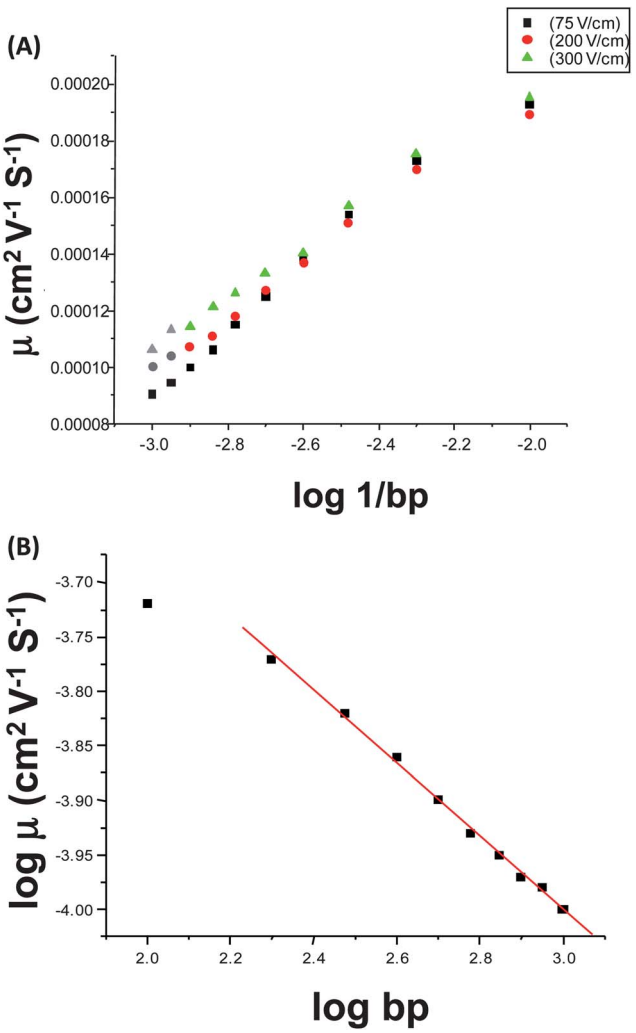

Fig. 3 (A) Mobility of DNA (μ) *versus* log 1/bp under different electric fields on the PeT microchip. DNA concentration: 25 ng μL^{-1}; electrokinetic injection: 300 V per 30 s; confocal laser-induced fluorescence detection system equipped with a 488 nm argon ion laser for excitation. Effective separation length was 34 mm from the injection point. (B) Linearity between log bp and log of mobility for DNA fragments between 200 and 1000 bp ($r^2 = 0.9974$) using 0.5% HEC as separation matrix and electric field of 200 V cm^{-1} for separation.

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is more convenient to minimize the effect of mobility variation from such fragments, low electric fields result in longer, undesirable analysis times. Hence, optimal DNA separation conditions require an applied electric field strength that provides a logical balance between separation efficiency, resolution and analysis time.

Linearity of DNA sizing

For a precise determination of the size of a DNA fragment within a particular size range, there must exist a linear relationship between the log of the number of base pairs and the log of mobility. Under ideal conditions, when r^2 is maximized for the whole extension ($r^2 = 1$), all fragments migrate consistent with a single mechanism of separation, *i.e.*, they exploit the same electromigration mechanism. In practice, when r^2 increases, one mechanism of separation dominates over others. Fig. 3B shows log μ *versus* log bp for fragments with 100 to 1000 bp under an electric field of 200 V cm^{-1}.

In Fig. 3B, it is clear that a linear relationship between log bp and log of mobility exists for fragments between 200 and 1000 bp. 'Reptation' is the predominant mechanism for fragments in this size range, and only the 100 bp fragment exhibits a deviation from this. The preferred mechanism of separation for short fragments follows the Ogston model.[23] In this case, the corresponding point of 100 bp was eliminated for the calculation of correlation coefficient (r^2), obtaining a value of 0.9974 for the region ranging from 200 to 1000 bp. The data are in agreement with those reported previously by our group, which found $r^2 =$ 0.9948 for fragments between 201 and 2036 bp for separations carried out on silica capillaries and HEC 140–160 kDa as separation matrix.[24]

Sequential injections on the same device with the same polymeric matrix

In this study, we evaluated the capability of the system to accommodate repeated, sequential injection/separation cycles without replacing the separation matrix. Commercial DNA analyzers based on microchips, such as Agilent 2100 Bioanalyzer, are able to analyze multiple samples on a single chip using a single load of separation matrix. A competitive system should be capable of performing repeated DNA separations on the same chip with a single chip preparation, thus reducing the cost per sample and time for batch analysis.

A series of injections of DNA ladder were carried out with the goal of determining the lifetime for use of a single sieving matrix in a PeT microchip. Fig. 2B shows the results of five consecutive injections of DNA ladder with 100–1000 bp fragments. The separations were reproducible for four consecutive injections in a PeT microchip loaded once with separation polymer, without noticeable changes in migration time or separation efficiency. With the fifth consecutive injection on the same matrix, changes in the resolution and migration time became apparent. This was a combination of sample depletion, low electroosmotic flow characteristic of PeT microchips,[10] and possible pH changes in the matrix. While low electroosmotic flow is key to allowing the PeT microchip to be used for DNA separation with sieving matrix without any surface pretreatment of the channel, the every same low electroosmotic flow (0.35×10^{-4} cm^2 V^{-1} s^{-1})[10] is responsible for the loss of resolution and lengthening of migration time after four injections. This feature is an advantage of this substrate material when compared to glass or PDMS chips[25] where the EOF needs to be suppressed.

Even though the PeT microchip is considered as disposable (because of its negligible cost), more than one run was carried out on a single device using the same separation matrix. The success of five consecutive separations without significant efficiency loss proved that it is possible to reuse the same PeT microchip for multiple injections. This contrasts with commercial systems using glass chips that are used for a single analysis. The great disadvantage is the high cost per chip (about US$40.00), and it is discarded after one run.

Robustness

In order to provide a robust platform for disposable chips, yet maintaining good separation quality and system flexibility, the PeT chips were filled with hydroxypropyl cellulose as opposed to the HEC used up to this point. Sanders *et al.* previously demonstrated that HPC polymer solution is a low-viscosity matrix for effective separation of DNA[25] and, thus, ideal for PeT chips. Although the lamination step seals both sheets of polyester together by means of the toner layer deposited between them, the mechanical resistance is limited; therefore, the less viscous the matrix the better. Additionally, this would provide insight into PeT chip versatility with other cellulosic matrices and different DNA ladders. Fig. 4 shows the electropherogram from a 100 bp DNA ladder from a different vendor (New England BioLabs). While keeping the chip geometry and configuration (with the exception that the detection spot was located at 35 mm from the injection point as opposed to 34 mm), the matrix was changed to 0.5% HPC in 0.1× TE buffer and the electric field was lowered to

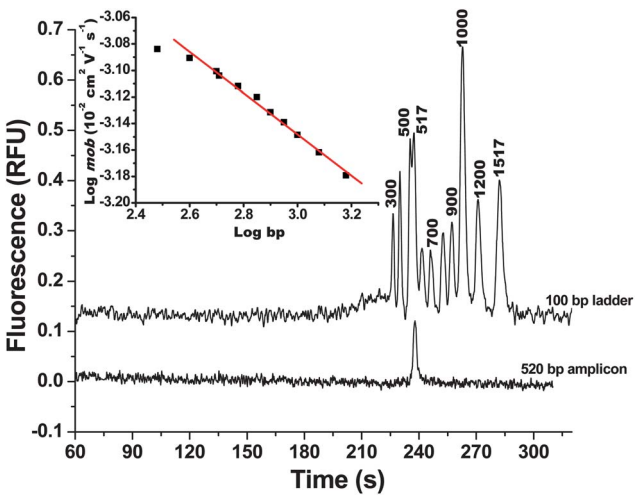

Fig. 4 Electropherograms showing the separation from a 100 bp DNA ladder and the separation of a 520 bp DNA fragment on PeT microchip. Experimental conditions: matrix: HPC 0.5% in 0.1× TE buffer; electrokinetic injection: 200 V cm^{-1} per 60 s; separation: 75 V cm^{-1} applied to the separation channel with 4 cm of total length; and detection at 3.5 cm from the injection point. Inset: sizing calibration for log μ *vs.* log DNA size in base pairs.

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75 V cm^{-1}, conditions that we defined as minimally affecting biased reptation (Fig. 3). The inset of Fig. 4 shows the linear regime up to 1500 pb with greater deviation for the shorter DNA fragments, such as 300 bp, in total agreement with separations obtained with HEC, with addition of superior efficiency (about 1 000 000 plates per m).

Analysis of a PCR product and sizing of DNA

Fig. 4 shows the electropherogram of DNA separation of the λ-phage DNA of 520 bp amplicon on the PeT microchip using the HPC polymer solution. Based on the plot of log of mobility *vs.* log bp (inset, Fig. 4), the calculation of the size of this amplicon was 520 ± 10 (n = 4), which is remarkable based on the fact that external size markers were not used to normalize the sizing. For comparison, the same sample was sized on the Bioanalyzer, and the amplicon sized as 518 ± 2 bp (n = 3), which defines the amplicon size by comparison with two size markers (15 and 1500 bp for the 1000 bp kit) using highly optimized separation conditions. The PeT system is, thus, able to separate and detect a DNA fragment produced by PCR, showing the potential for applicability of the system to detection with real-world samples, and also for the integration of separation with PCR amplification.

Conclusions

Gel electrophoresis is a critical analysis step in many genetic assays, and there is a strong demand for fast, low-cost, and high-throughput DNA electrophoresis technology. The PeT microchips are demonstrated here to be capable of separating DNA ladders (between 100 and 1500 bp) with reasonable resolution, efficiency, and speed. We successfully demonstrated that the low EOF on PeT chips is suitable for carrying out DNA separations with satisfactory run-to-run repeatability. We showed that the major contribution of low efficiency and resolution (relative to glass microchips) arises from roughness of the wall and the larger width of the injection plug. Such parameters will be studied in more detail for improving the separation, along with other parameters such as matrix separation, electric field strength, channel dimensions, and ionic composition of the running buffer. Despite the need for further optimization, we present PeT microdevices as low-cost, easy-to-fabricate microchips that do not require any channel conditioning or surface modification, and are compatible with HEC and HPC diluted polymer solutions for DNA sizing.

PeT devices can clearly be classified as disposable microfluidic devices, which will obviously be of interest for potential use in point-of-care applications as well as in clinical analysis. The potential for PeT microchips to facilitate fast and low-cost DNA separations is particularly important in light of our recent report showing solid phase extraction of DNA followed by PCR on PeT microchips. The demonstrated capabilities of PeT chips for DNA separation are the last step of DNA analysis necessary to produce a fully integrated PeT microchip. The integration of all such steps for DNA analysis in a disposable PeT device is currently under exploration, where the direct-printing technology will allow for the construction of a multifunctional integrated microdevice that is unprecedented in terms of cost and performance for genetic analysis.

Abbreviations

PeT	Polyester–toner
HEC	Hydroxyethylcellulose
HPC	Hydroxypropylcellulose
TE	Tris–EDTA

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