analytical chemistry

Article

pubs.acs.org/ac

Droplet Interfaced Parallel and Quantitative Microfluidic-Based Separations

Sammer-ul Hassan,[†] Hywel Morgan,[‡,§] Xunli Zhang,[†,§] and Xize Niu*[,†,§]

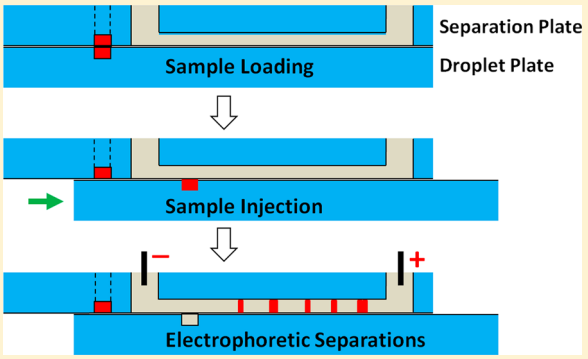
[†]Faculty of Engineering and the Environment, University of Southampton, Southampton, U.K. SO17 1BJ

[‡]Faculty of Physical Sciences and Engineering, University of Southampton, Southampton, U.K. SO17 1BJ

[§]Institute for Life Sciences, University of Southampton, Southampton, U.K. SO17 1BJ

Supporting Information

ABSTRACT: High-throughput, quantitative, and rapid microfluidic-based separations has been a long-sought goal for applications in proteomics, genomics, biomarker discovery, and clinical diagnostics. Using droplet-interfaced microchip electrophoresis (MCE) techniques, we have developed a novel parallel MCE platform, based on the concept of combining the Slipchip principle with a newly developed "Gelchip". The platform consists of two plastic plates, with droplet wells on one plate and separation channels with preloaded/cured gel in the other. A single relative movement of one plate enables generation and then loading of multiple sample droplets in parallel into the separation channels, allowing electrophoretic separation of biomolecules in the droplets in parallel and with high-throughput. As proof of concept, we demonstrated the separation of 30 sub-nL sample droplets containing fluorescent dyes or DNA fragments.



Electrophoresis in all its embodiments is a powerful analytical technique which has been applied to resolve complex mixtures containing DNA, proteins, and other chemical or biological species.[1−4] The development of microfabrication techniques has led to further miniaturization of electrophoresis known as microchip electrophoresis (MCE), which offers particular advantages including ultrasmall volume sample consumption, integration with other "lab-chip" processes or functions such as extraction, purification, washing, mixing, and sample concentration.[5,6] As a result, MCE has been used in a variety of applications, e.g., to analyze biomolecules in blood,[7] saliva,[8] tear,[9] dialysate,[10] and islets.[11]

The majority of MCE and capillary electrophoresis (CE) technologies have used one of the two common sample injection methods, i.e., electrokinetic or hydrostatic injection. In the former, the sample injection may introduce bias as different analytes have different electrophoretic mobilities;[12,13] therefore, the injected sample may not reflect the concentration and composition of the original sample.[4] Hydrostatic sample injection technique has many difficulties, e.g., in controlling the flow in the small microchannels, and has limited throughput.[14] To analyze samples in parallel, microfluidic chips have been fabricated that consist of arrays of microchannels.[12,15−19] However, these devices use the same sample injection methods listed above. Pan et al. has recently shown an elegant method of parallel separation in free-standing gel strips with 96 wells, and this could lead to high throughput and quantitative analysis with a low running cost.[20]

Droplet-based microfluidics has immerged as a powerful tool, and the technique can encapsulate biological samples in discrete droplets, enabling manipulation and analysis in a high throughput format.[21−24] Subnanoliter sample droplets can be generated in a microfluidic chip or collected from a bioreactor, an upstream separation column, or even from a tissue environment.[25−27] These discrete sample droplets can be further analyzed by electrophoresis by injecting them into a separation channel.[28,29] Such droplet-interfaced systems have been shown to be effective for sample injection and offer many other advantages including ultrasmall volume consumption, no sample waste, quantitative analysis without bias, simple device setup, and no electric field switching. Interestingly such systems also reintroduce CE as a powerful analytical tool to resolve complex mixtures within microdroplets.[10,29] However, in these droplet-interfaced separations, sample droplets are mostly analyzed in serial that limits throughput. Jian[30] has addressed this shortcoming by expanding an original single separation channel to three, but further multiplexing requires complicated droplet manipulation, which may not be feasible.

The Slipchip, first developed by Ismagilov's group,[31] is an effective new method for parallel droplet operations. It is a simple device consisting of two plates with small wells fabricated in each. The wells can be filled with different reagents and upon sliding one layer relative to the other,

Received: December 17, 2014
Accepted: March 16, 2015

DOI: 10.1021/ac504695w
Anal. Chem. XXXX, XXX, XXX−XXX

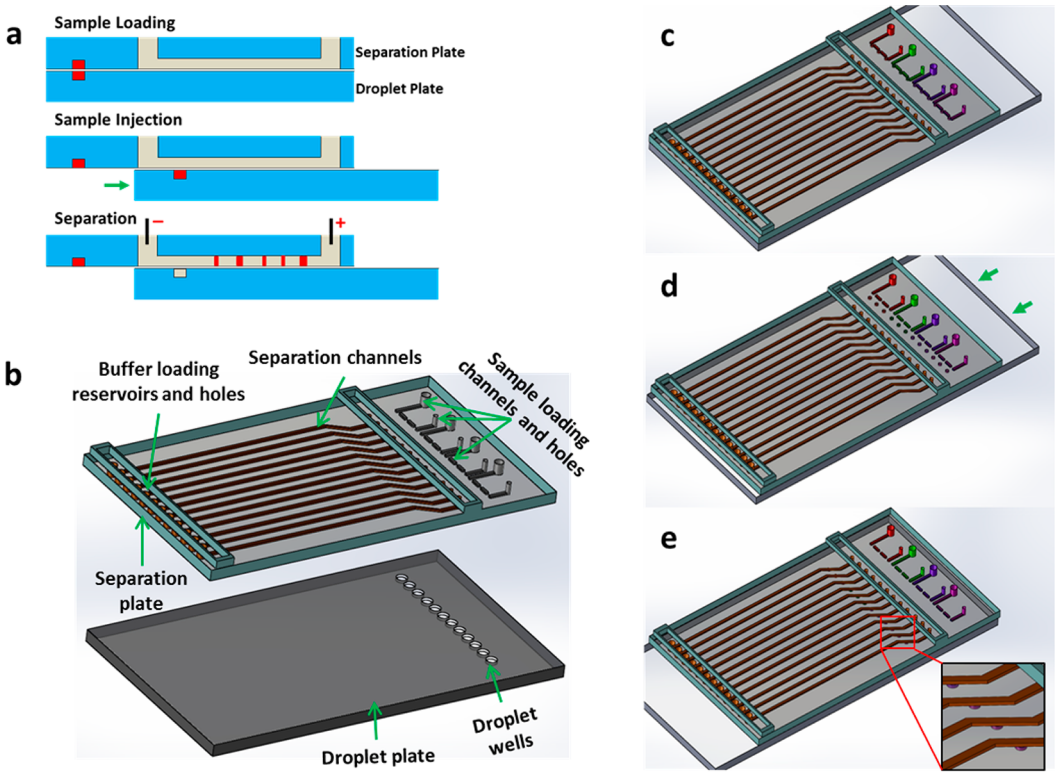


Figure 1. 3D Schematic showing the working principle of the device (dimensions are not drawn to scale). (a) Schematic view of droplet generation, injection, and sample separation. The arrow indicates the movement direction of the droplet plate. (b) Schematic of the separation plate and droplet plate. (c) Initial position of chips after assembly and loading samples. (d) Slipping bottom layer to generate droplets (arrows show the movement direction of the droplet plate). (e) Injection of droplets into the separation channels.

various operations can be implemented such as generation of sample droplets or fusion of droplets to initiate chemical reactions. We[32] and later Shujun et al.[33] demonstrated that this Slipchip format has the potential to be used for separation science, e.g., for segmenting separated samples after isoelectric focusing (IEF) into microdroplets to avoid any sample remixing during postseparation sample collection.

Herein, we combine the concepts of the Slipchip and MCE and develop a novel device that can achieve parallel droplet interfaced separations, by loading droplets into separation gel/medium with the "slipping" function. With this sample loading method, the entire volume of the sample droplets can be separated. Therefore, it offers the ability to quantify biomolecules in sample droplets.

EXPERIMENTAL SECTION

Materials. Fluorescein 5(6)-isothiocyanate (FITC), fluorescein, 5-carboxyfluorescein, eosin Y, tris borate buffer (TBE), and agarose powder were obtained from Sigma-Aldrich (Dorset, U.K). Poly(ethylene oxide) (PEO, 500 kDa) was purchased from Avocado Research Chemicals Ltd. (Lancashire, U.K.). Solutions of 30% (w/v) acrylamide/bis(acrylamide), cross-linker (TEMED), initiator (ammonium persulfate), and sodium dodecyl sulfate (SDS) were also purchased from Sigma-Aldrich (Dorset, U.K). DNA ladder (Mapmarker FAM labeled) was purchased from BioVentures Inc. (Murfreesboro).

Sample Preparation. Commercially available reagents were bought and used without further purification. Fluorescein 5(6)-isothiocyanate (FITC), fluorescein, 5-carboxyfluorescein, and eosin Y were dissolved in 0.1× TBE at a stock concentrations of 300 μM. DNA samples were prepared by mixing standard Mapmarker ladder with formamide at equal volumes and diluted with 1× TBE to achieve 10× diluted standard sample. The sample mixture was predenatured at 95 °C for 2 min and snap-cooled on ice prior to loading to the sample channels.

Microchip Fabrication and Preparation. The microfluidic chips used for all experiments were fabricated by a precise micromilling in poly(methyl methacrylate) (PMMA) sheets using an LPKF micromilling machine (ProtoMat-S100). The separation channels (150 μm × 200 μm width × depth, 7 cm long), via holes (300 μm diameter), cathode and anode reservoirs were milled on the top plate, while microwells for sample droplets (150 μm × 200 μm × 200 μm width × length × depth) were milled on the bottom plate. The chip surface was rendered smooth using chemical reflow.[34] Briefly, a small container was filled with chloroform and the microchip was placed on top of the container with all the channels exposed to chloroform vapor. The distance between the microchip and the chloroform layer was kept at 5 mm and an exposure time of 3−4 min was found to be suitable for reflow of the PMMA surface. A longer exposure time of more than 4 min was found to damage the channels and cracks appeared on the surface. Chloroform is a hazardous material and the vapors can cause acute toxicity, irritation, or carcinogenicity. Therefore, the reflow process was performed in a fumehood and personal protective equipment were used. Both of the MCE chips were treated with Duxback (Duxback Ltd.) and heated at 65 °C for 10 min in an oven to evaporate the solvent making the PMMA surface hydrophobic. Before use, 30−50 μL of fluorinated oil (FC-40) was spread over the chip to wet the surface thus preventing leakage of sample from the wells during slipping.

DOI: 10.1021/ac504695w
Anal. Chem. XXXX, XXX, XXX−XXX

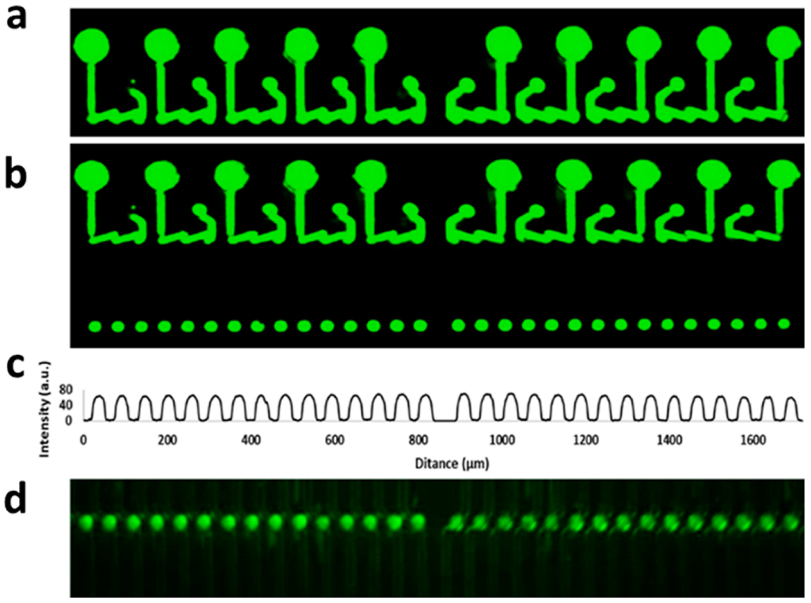


Figure 2. Droplet generation and injection. (a) Fluorescein filled in the sample loading channels. (b) Droplets generated after slipping the chip. (c) Fluorescence intensity profile of the droplets. (d) Droplets injected into separation channels.

The sample and buffer were loaded into the microchip by a pipet.

f1 **Microchip Design and Operation.** Figure 1a shows the schematic diagram of the chip design and operation. The chip consists of separation channels, sample loading channels, buffer reservoirs together with holes on the top plate and droplet wells on the bottom plate. Henceforth, the top plate is referred to as the separation plate and the bottom plate as the droplet plate.

After fabrication with PMMA material, the plate surfaces were smoothed by reflowing with chloroform and coating with Duxback. This produced surfaces with excellent optical clarity and hydrophobicity (see Figure S1 in the Supporting Information). To facilitate "nonmicrofluidic users", we have also developed protocols that precure separation gels (e.g., agarose, polyacrylamide) in open channels forming a "Gelchip" that can be prepared in batch and used off-the-shelf. The separation plate was joined to the droplet plate in such a way that the sample loading channels were connected with droplet wells forming zigzag channels as shown in Figure 1a.

To seal the microchannels and minimize sample sticking on the surface or any leakage, fluorinated oil (FC-40) was added at the interface, especially covering the areas between the droplet generation and the sample loading channels. The oil also lubricates the two plates and minimizes surface friction during movement of the plates. The two chip halves were clamped together using magnets on opposite sides to ensure a tight contact of the two plates.[32] After assembly, the sample mixture was loaded into the sample loading channels via inlet holes (0.8 mm diameter) that were fitted with the end of a pipet tip. The separation buffer (TBE/Tris-Ches) was then loaded to the two buffer reservoirs connecting to both ends of the separation channels. The 0.5 mm diameter platinum wire electrodes were placed in each reservoir to provide a uniform electric field to all of the parallel channels. Noted in certain separation modes when liquid sieving matrixes are required, such as poly(ethylene oxide) (PEO) solution for capillary gel electrophoresis (CGE) or other capillary zone electrophoresis (CZE) buffers, the separation channels were left empty during chip assembly. Separation matrixes were then added to the channels before sample loading, by gentle liquid pumping to the inlet holes with a peristaltic pump at a flow rate of 3 μL/min. The droplet plate was then moved by a micrometer connected to the droplet plate from its initial position; thereby generating droplets in each of the wells (Figure 1d,e). During this process, sample droplets were first generated from the sample channels and further moved to overlap with the separation channels. Finally a dc electric field was applied across the reservoirs and migrating the sample molecule toward the opposite charge end performing electrophoresis separations.

Droplet Generation and Injection. Droplet generation and injection were initially calibrated by loading fluorescein dye solution into the sample loading (zigzag) channels connected to the droplet wells (Figure 2a). After slipping the chip, each f2 sample produced 3 repeat droplets, as shown in Figure 2b. Fluorescence intensity profiles of the generated droplets gives information on the reproducibility of the droplet area (Figure 2c). The percent relative standard deviation (% RSD) for all of the 30 droplets in more than 3 runs was <3%. In Figure 2d, droplets were moved to be in contact with the separation channels. There was no surfactant added into the oil; therefore, the aqueous droplet immediately merged with the gel and sample molecule started to diffuse into the separation channels. Confocal imaging taken 30 s after droplet merging showed that the fluorescent molecules had already diffused into the separation gel (Figure S6 in the Supporting Information). Droplet interfacing is a new approach to sample loading, relying on segmentation, and loading of sample droplets; therefore, appropriate oil is needed to prevent unwanted droplet breakup, sample leakage, or surface contamination. The FC-40 oil membrane trapped in between the two plates kept the aqueous sample droplets inside the droplet wells while it was moved toward the separation channels. However, the detergent SDS was found to destabilize the oil–water interface (FC-40 oil and TBE buffer combination) causing severe sample loss into the interface of the two plates; therefore, we do not recommend adding SDS into the sample for this method.

DOI: 10.1021/ac504695w
Anal. Chem. XXXX, XXX, XXX–XXX

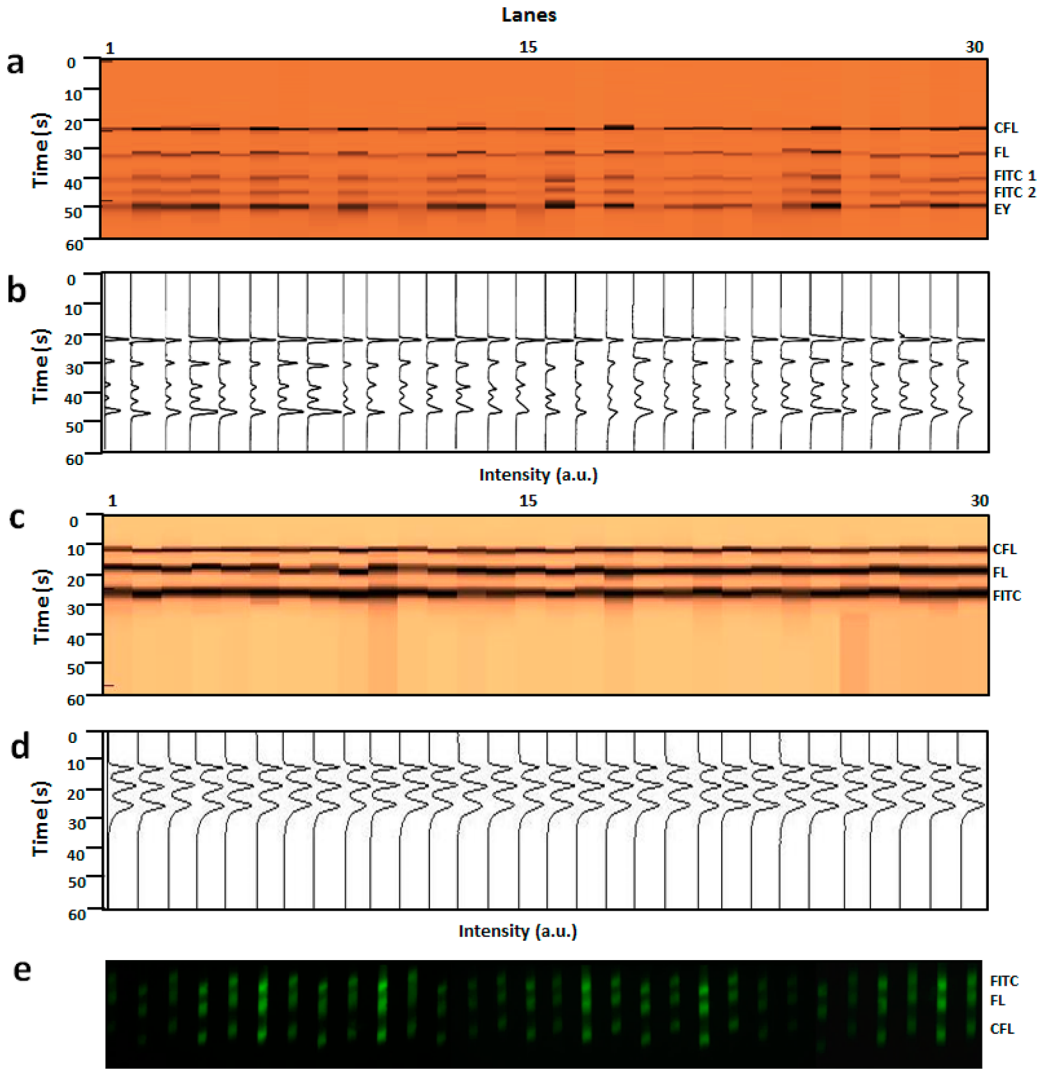


Figure 3. Separation of fluorescent dyes in PEO gel (a, b) and agarose gel (c, d, e). (a) Pseudo gel plot from a PEO gel separation for sample mixture (Eosin Y, FITC 1, FITC 2, fluorescein, and 5-carboxyFL). (b) Corresponding electropherograms. Field strength, 90 V/cm; detection point, 3.5 cm; separation medium, 1.5% PEO gel. (c) Pseudo gel plot from an agarose gel separation for sample mixture (FITC, fluorescein, and 5-carboxyFL). (d) Corresponding electropherograms. (e) Microscope snapshot of the separated bands. Field strength, 80 V/cm; detection point, 6 mm; separation medium, 2% agarose gel.

RESULTS AND DISCUSSION

Separation reproducibility was determined by separating fluorescent dyes in the microchannels. In this experiment, all the sample droplets contained the same sample mixture. FITC, fluorescein, eosin Y, and 5-carboxyfluorescein at concentrations of 25, 10, 144, and 13.5 μM, respectively, were prepared in 0.1× TBE buffer and separated using 1.5% PEO gel (500 kDa). The dyes were negatively charged at pH 8.4 and migrated toward the anode when an electric field (90 V/cm) was applied. The bands of separated dyes were detected at a distance of 3.5 cm from the sample injection point. The pseudo gel plot and representative electropherograms were drawn in Figure 3a,b, from the fluorescent intensity data collected from recorded videos. The electropherogram was realigned according to the peaks from 5-carboxyfluorescein as an internal standard. It is clearly seen that baseline separation was achieved and the separation was completed in 60 s. The apparent number of theoretical plates, an indicator of maximum separation efficiency of a separation column/channel is given by $N = 5.54[t_r/W_{t_{1/2}}]^2$, where $W_{t_{1/2}}$ is the width of the peak at half of the height (expressed in terms of time) and t_r is the retention time of the separated molecule, both were measured from recorded videos using a homemade Matlab (Mathworks) program. Theoretical plates were calculated to be 7560 at a distance of 3.5 cm. This value is 1 order of magnitude less than for glass chip-based separations.[35] This lower number of theoretical plates can be attributed to three main factors. First, there was molecular diffusion at the injection point and in the separation channels. The diffusion was more obvious for small fluorescent molecules than larger biomolecules (as shown in the later DNA separation with higher plate numbers); second, separation conditions such as buffer/gel concentrations, surface coating, and electric field strength have not been optimized in this study; and third, the droplet volume (800 pL) used here was larger compared to the 10 ms injection time in a cross-piece injections.[35] The separation reproducibility was also calibrated in the precasted agarose gel (2% agarose), as shown in Figure 3c,d. The theoretical plates were calculated to be 1890 at a distance of 6 mm. Supplementary Movie 3 in the Supporting

DOI: 10.1021/ac504695w
Anal. Chem. XXXX, XXX, XXX−XXX

Information provides a short recording for the separation of these dyes in 15 channels filled with agarose gel.

We further determined the effect of droplet sizes on the separation efficiency. Four different droplet wells were fabricated with different well depths to generate droplets with volumes of 320, 800, 1280, and 1750 pL as shown in Figure S4a,b in the Supporting Information. FITC and fluorescein were separated within these four different droplet sizes (Figure S4c,d in the Supporting Information) and separation resolution (SR) was determined. It was found that the SR decreases with the increasing droplet volume. The bands were highly resolved for the smallest droplet volume (Figure S5e in the Supporting Information). However, there are slight deviations from a linear fit which could be due to the differences in droplet size, variations in electric field strength in different channels, and wettability of the channels which affects aqueous droplet merging. The theoretical plates achieved by this separation are 2220 (corresponding to the smallest droplet) to 1480 (the largest droplet) at a distance of 8 mm.

This method of droplet-interfaced separation allows for whole sample injection from the droplets to the separation channels without any sample waste. Therefore, quantitative analysis of analytes within the sample mixture can be achieved. For each sample mixture, the chip produces multiple sample droplets (three in this setup). The separation results can be compared to provide a standard derivation, as is generally required in a biochemical analysis. Since these sample repeats are analyzed in parallel, no extra separation time is required in our system. To validate this method, mixtures of samples were prepared with fixed concentrations of 5-carboxyluorescein (9 μM) and fluorescein (50 μM) and varying the FITC concentration from 0 to 50, 100, 150, 200, and 250 μM. Each sample was injected into one sample channel to produce three droplet copies, which were then separated in corresponding channels and the results are shown in Figure 4. A program written in Matlab was used to extract the peak areas, which were further normalized using the peak area of 5-carboxyfluorescein as an internal standard. The change in peak areas has a linear correspondence (3.6% RSD) to the original sample concentrations as illustrated in Figure 4c.

DNA sizing and protein separation are important applications of gel electrophoresis in biochemistry, forensics, and immunoassays. Here DNA ladders from 50, 100, 150, 200, 300, 400, 500, and 600 bp were separated to assess the performance of the device with PEO gel, which is a well-studied sieving matrix for separating DNA fragments ranging from 25bp to over 2000bp.[3,36] With the gel loading methods described in the Experimental Section, different PEO (500 kDa) concentrations ranging from 0.5 to 3% were successfully loaded to separation channels and the DNA ladder was best separated in 2.5% PEO gel in the device. Figure 5 shows the detection at a distance of 13 mm from the point of injection, and the separation was completed within 120 s. The number of theoretical plates were calculated to be 79 800, comparable with the other microchip based DNA separations using microchips,[37] which is 1 order of magnitude higher than previously achieved theoretical plates using our droplet interfaced microfluidic chips.[29]

CONCLUSION AND DISCUSSION

In this paper, we have developed a novel droplet-interfaced microchip electrophoresis device that provides parallel and quantitative separations of analytes from subnano liter droplets. The chip contains precured agarose or polyacrylamide gel,

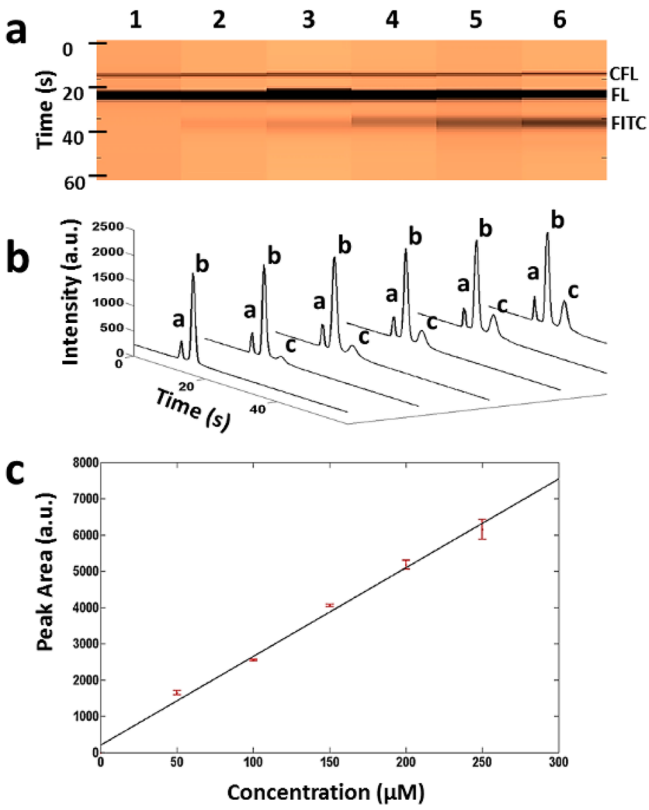


Figure 4. Quantitative analysis of sample droplets. (a) Pseudo gel plot. (b) Electropherograms. (c) Standard curve for peak area vs concentrations of FITC. FITC concentrations from left to right are 0, 50, 100, 150, 200, 250 μM. Field strength, 80 V/cm; detection point, 8 mm; separation medium, 2% agarose gel.

hence the name "Gelchip", and can be used off-the-shelf. A liquid separation medium (e.g., PEO) can also be loaded into the channels in situ; therefore, the device supports a wide range of separation methods. Although in our initial study, each channel was loaded with a homogeneous gel, advanced separation abilities could be added using gels with gradients[38] or forming preconcentration zones within the channels[39] or online labeling by curing derivatization dyes into the gel.[40]

In the prototype, sample droplets were generated by one slip of the chip and the sample breaks up in the zigzag channels to form droplets. Droplets could also be pregenerated by the other droplet generation or collection methods or devices and trapped in these zigzag channels/wells for CE analysis. While on-chip PCR and immunoassay functions have been demonstrated for Slipchips, multiple step assays could be integrated leading to a self-contained complex diagnostic device. After separation, the Slipchip plates can be detached and the gel can be used for other analytical methods or exposed to other chemicals. This could facilitate postseparation staining and destaining or MALDI MS that are under study. The device is user-friendly and has the potential to be applied for DNA sizing, peptide and protein separation, or immunoassays in a high-throughput format using minute amounts of sample. With future improvement in the detection capacity and sensitivity of the system, the device has the potential to be applied for high-resolution analysis of complex mixtures with hundreds of droplets or for 2D separations of serum proteins, quantitative immunoassays, or Western blot analyses.

DOI: 10.1021/ac504695w
Anal. Chem. XXXX, XXX, XXX−XXX

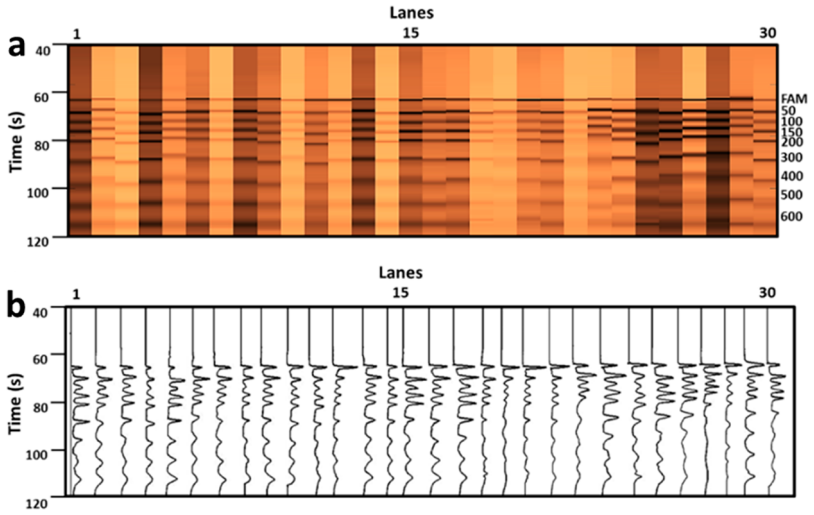


Figure 5. Separation of 50, 100, 150, 200, 300, 400, 500, and 600 bp DNA ladder (BioVentures Mapmarker FAM labeled), (a) pseudo gel plot of DNA fragments. (b) Electropherograms. Field strength, 100 V/cm; detection point, 1.3 cm; separation medium, 2.5% PEO.

■ ASSOCIATED CONTENT

Supporting Information

Fabricated chip plates, gel curing protocol, microchip separation and data analysis, calibration of the relationship between droplet volume and separation resolution, and supplementary movies (3D movie of droplet injection, fluorescein movie of droplet injection, and separation video). This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: x.niu@soton.ac.uk.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was partially supported by the Engineering and Physical Sciences Research Council UK (Grant EP/M012425/1), and we thank Mr. Junjun Lei for help on COMSOL Simulation. H.M. would like to acknowledge the Royal Society for funding.

■ REFERENCES

(1) Ostergaard, J.; Jensen, H. *Anal. Chem.* **2009**, *81*, 8644−8648.
(2) Mosca, A.; Paleari, R.; Mosca, L.; Marcello, A.; Vercellati, C.; Zanella, A. *Clin. Biochem.* **2009**, *42*, 1859.
(3) Pereira, F.; Hassard, S.; Hassard, J.; deMello, A. *Electrophoresis* **2009**, *30*, 2100−2109.
(4) Huang, X. H. C.; Quesada, M. A.; Mathies, R. A. *Anal. Chem.* **1992**, *64*, 2149−2154.
(5) Haeberle, S.; Zengerleab, R. *Lab Chip* **2007**, *7*, 1094−1110.
(6) Roddy, E. S.; Xu, H.; Ewing, A. G. *Electrophoresis* **2004**, *229*−*42*, 229.
(7) Koutny, L. B.; Schmalzing, D.; Taylor, T. A.; Fuchs, M. *Anal. Chem.* **1996**, *68*, 18−22.
(8) Herr, A. E.; Hatch, A. V.; Throckmorton, D. J.; Tran, H. M.; Brennan, J. S.; Giannobile, W. V.; Singh, A. K. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 5268−5273.
(9) Karns, K.; Herr, A. E. *Anal. Chem.* **2011**, *83*, 8115−8122.
(10) Wang, M.; Roman, G. T.; Perry, M. L.; Kennedy, R. T. *Anal. Chem.* **2009**, *81*, 9072−9078.
(11) Dishinger, J. F.; Reid, K. R.; Kennedy, R. T. *Anal. Chem.* **2009**, *81*, 3119−3127.
(12) Harrison, D. J.; Manz, A.; Fan, Z. H.; Ludi, H.; Widmer, H. M. *Anal. Chem.* **1992**, *64*, 1926−1932.
(13) Ermakov, S. V.; Jacobson, S. C.; Ramsey, J. M. *Anal. Chem.* **2000**, *72*, 3512−3517.
(14) Tabuchi, M.; Kuramitsu, Y.; Nakamura, K.; Baba, Y. *Anal. Chem.* **2003**, *75*, 3799−3805.
(15) Yang, S.; Liu, J.; Lee, C. S.; DeVoe, D. L. *Lab Chip* **2009**, *9*, 592−599.
(16) Emrich, C. A.; Medintz, I. L.; Chu, W. K.; Mathies, R. A. *Anal. Chem.* **2007**, *79*, 7360−7366.
(17) Gaunt, T. R.; Hinks, L. J.; Rassoulian, H.; Day, I. N. M. *Nucleic Acids Res.* **2003**, *31* (9), e48−e48.
(18) Paegel, B. M.; Emrich, C. A.; Wedemayer, G. J.; Scherer, J. R.; Mathies, R. A. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 574−579.
(19) Bousse, L.; Mouradian, S.; Minalla, A.; Yee, H.; Williams, K.; Dubrow, R. *Anal. Chem.* **2001**, *73*, 1207−1212.
(20) Pan, Y.; Duncombe, T. A.; Kellenberger, C. A.; Hammond, M. C.; Herr, A. E. *Anal. Chem.* **2014**, *86*, 10357−10364.
(21) Chiu, D. T. *TrAC, Trends Anal. Chem.* **2003**, *22*, 528−536.
(22) Niu, X.; de Mello, A. J. *Biochem. Soc. Trans.* **2012**, *40*, 615−623.
(23) Utada, A. S.; Lorenceau, E.; Link, D. R.; Kaplan, P. D.; Stone, H. A.; Weitz, D. A. *Science* **2005**, *308*, 537−541.
(24) Song, H.; Chen, D. L.; Ismagilov, R. F. *Angew. Chem., Int. Ed* **2006**, *45*, 7336−7356.
(25) Niu, X. Z.; Zhang, B.; Marszalek, R. T.; Ces, O.; Edel, J. B.; Kluga, D. R.; deMello, A. J. *Chem. Commun.* **2009**, *41*, 6159−6161.
(26) Draper, M. C.; Niu, X. Z.; Cho, S.; Jarnes, D. I.; Edel, J. B. *Anal. Chem.* **2012**, *84*, 5801−5808.
(27) Edgar, J. S.; Milne, G.; Zhao, Y. Q.; Pabbati, C. P.; Lim, D. S. W.; Chiu, D. T. *Angew. Chem., Int. Ed* **2009**, *48*, 2719−2722.
(28) Edgar, J. S.; Pabbati, C. P.; Lorenz, R. M.; He, M. Y.; Fiorini, G. S.; Chiu, D. T. *Anal. Chem.* **2006**, *78*, 6948−6954.
(29) Niu, X.; Pereira, F.; Edel, J. B.; de Mello, A. J. *Anal. Chem.* **2013**, *85*, 8654−8660.
(30) Pei, J.; Nie, J.; Kennedy, R. T. *Anal. Chem.* **2010**, *82*, 9261−9267.
(31) Du, W.; Li, L.; Nichols, K. P.; Ismagilov, R. F. *Lab Chip* **2009**, *9*, 2286−2292.
(32) Zhao, Y.; Pereira, F.; de Mello, A. J.; Morgan, H.; Niu, X. *Lab Chip* **2014**, *14*, 555−561.
(33) Wang, S.; Chen, S.; Wang, J.; Xu, P.; Luo, Y.; Nie, Z.; Du, W. *Electrophoresis* **2014**, *35*, 2528−2533.
(34) Ogilvie, I. R. G.; Sieben, V. J.; Floquet, C. F. A.; Zmijan, R.; Mowlem, M. C.; Morgan, H. *J. Micromech. Microeng.* **2010**, *20*, 065016.

DOI: 10.1021/ac504695w
Anal. Chem. XXXX, XXX, XXX−XXX

426 (35) Roman, G. T.; Wang, M.; Shultz, K. N.; Jennings, C.; Kennedy,
427 R. T. *Anal. Chem.* **2008**, *80*, 8231−8238.
428 (36) Tseng, W. L.; Chang, H. T. *Electrophoresis* **2001**, *22*, 763.
429 (37) Kang, C.; Back, S. K.; Song, I.; Choi, B.; Chang, J.; Cho, K.;
430 Kim, Y. *Bull. Korean Chem. Soc.* **2006**, *27*, 519−523.
431 (38) Hou, C.; Herr, A. E. *Anal. Chem.* **2010**, *82*, 3343−3351.
432 (39) Huang, H.; Xu, F.; Dai, Z. *Electrophoresis* **2005**, *26*, 2254−2260.
433 (40) Jin, L. J.; Giordano, B. C.; Landers, J. P. *Anal. Chem.* **2001**, *73*,
434 4994−4999.

DOI: 10.1021/ac504695w
Anal. Chem. XXXX, XXX, XXX−XXX