Integrated microfluidic chip for rapid DNA digestion and time-resolved capillary electrophoresis analysis

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An integrated microfluidic chip is proposed for rapid DNA digestion and time-resolved capillary electrophoresis (CE) analysis. The chip comprises two gel-filled chambers for DNA enrichment and purification, respectively, a T-form micromixer for DNA/restriction enzyme mixing, a serpentine channel for DNA digestion reaction, and a CE channel for on-line capillary electrophoresis analysis. The DNA and restriction enzyme are mixed electroosmotically using a pinched-switching DC field. The experimental and numerical results show that a mixing performance of 97% is achieved within a distance of 1 mm from the T-junction when a driving voltage of 90 V/cm and a switching frequency of 4 Hz are applied. Successive mixing digestion and capillary electrophoresis operation clearly present the changes on digesting ϕ x-174 DNA in different CE runs. The time-resolved electropherograms show that the proposed device enables a ϕ x-174 DNA sample comprising 11 fragments to be concentrated and analyzed within 24 min. Overall, the results presented in this study show that the proposed microfluidic chip provides a rapid and effective tool for DNA digestion and CE analysis applications. © 2012 American Institute of Physics. [doi:10.1063/1.3654950]

I. INTRODUCTION

Microfluidic devices have had a substantial impact on clinical analysis practices and have found widespread use in the food and chemical industries.^{1–8} Typically, microfluidic chips are used to perform such tasks as sample pre-treatment and injection, species mixing, polymerase chain reaction, and cell/particle separation and counting.^{9–16} The small flow channels within microfluidic systems increase the surface to volume ratio and are advantageous in many applications. However, the small characteristic size of the channels constrains the flow to the low Reynolds number regime. As a result, turbulent flow does not occur and species mixing is achieved as a result of diffusion alone. Consequently, obtaining a satisfactory mixing performance requires the use of extended flow channels and a prolonged retention time. However, neither requirement is compatible with the overriding goals of microfluidic systems design, namely, device miniaturization and a rapid throughput. Thus, efficient mixing schemes are urgently required.

Developing simple and reliable mixing methods is of great importance in improving the performance of microfluidic systems. Microfluidic devices incorporating micromixers and utilizing electrokinetic forces to drive the sample fluid are used in a wide variety of bio-analytical applications, including DNA restriction, multiple sample injection, sample extraction, and controlled fraction mixing.^{17–23} Yan *et al.*²⁴ presented a method for enhancing the performance of

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electrokinetically driven T-form micromixers by means of a frequency-modulated electric field and channel geometry effects. The results show that for a T-mixer of 10 mm mixing length, utilizing frequency modulated electric field and channel geometry effects can increase the mixing efficiency from 50% to 90%. Xu *et al.*²⁵ examined the thermal mixing characteristics of two miscible fluids in a T-form micromixer. It was shown that heat transfer between the two species occurred in both the T-junction and the main mixing channel, where the relative amount of heat transferred in each region was determined principally by the flow rate ratio of the two species.

Recently, numerous groups have investigated methods for integrated microfluidic devices^{26–35} for wide application in the fields of chemical and biological analyses. Kamholz *et al.*³⁶ incorporated a basic T-form micromixer within a device designed to measure the analyte concentrations of a continuous flow. Lin *et al.*³⁷ presented a rapid microfluidic mixer in which a freeze-quenching technique was used to trap the meta-stable intermediates produced during rapid chemical or biochemical reactions. Fu and Lin³⁸ presented a novel DNA digestion system in which the DNA and the restriction enzyme were mixed via fixed and periodic switching DC electric fields. Hong *et al.*³⁹ presented a rapid and straightforward approach for methanol concentration and detection using an integrated microfluidic chip patterned using a commercially available CO₂ laser scribe. Chang *et al.*⁴⁰ presented an integrated microfluidic chip, which integrates micropumps, a micromixer and a micro temperature module in a three-dimensional structure to automate the entire process of the extraction of the mtDNA. Kato *et al.*⁴¹ designed a polymer-based multilayer microchip used for vacuum distillation with the objective of using the device to successfully make methanol/water separation. The microchip included a cooling channel, a separated liquid phase channel, and a channel for vapor phase by an incorporated microporous polytetrafluoroethylene (PTFE) membrane. Wang *et al.*⁴² presented an integrated microfluidic system for detecting viruses from tissue samples by means of a targeted ribonucleic acid (RNA) extraction process followed by reverse transcription loop-mediated isothermal amplification (RT-LAMP). Hou *et al.*⁴³ presented a three-dimensional disposable integrated microfluidic chip for glucose concentration detection. The chip comprises a four-layer polymethylmethacrylate (PMMA) structure and incorporates a double parallel connection micro-mixer and a T-type outlet microchannel and is fabricated using a CO₂ laser ablation system and a hot-press bonding technique. Wu *et al.*⁴⁴ presented an integrated microfluidic system capable of isolation, counting, and sorting of hematopoietic stem cells (HSCs) from cord blood in an automatic format by utilizing a magnetic-bead-based immunoassay. The microfluidic chip is comprised of three functional modules including cell isolation, cell counting, and cell sorting modules.

This paper proposes an integrated microfluidic device for rapid DNA digestion and time-resolved capillary electrophoresis (CE) analysis. The chip integrates two gel-filled chambers for DNA enrichment and purification, respectively, a T-form micromixer for DNA/restriction enzyme mixing, a serpentine channel for DNA digestion reaction, and a CE channel for on-line capillary electrophoresis analysis. The proposed system is operated using a single power supply and has the advantages of simplicity, stability, and reliability. The mixing performance of the T-form micromixer is evaluated both experimentally and numerically. In addition, the DNA digestion and CE analysis performance of the proposed integrated device are evaluated experimentally using a standard bio-reaction system comprising Hae III restriction enzyme and ϕ X-174 DNA.

II. CHIP DESIGN AND WORKING PROCESS

Figure 1 presents a functional block diagram of the proposed device. Briefly, the DNA sample is concentrated within a gel-filled chamber and is then injected into a T-form micromixer together with a restriction enzyme. The two species are mixed by a switching DC electric field and then flow through a serpentine channel where digestion of the DNA sample takes place. The digested sample is passed through a gel-filled chamber, and the DNA fragments are then injected, separated, and detected on-line in a cross-type CE channel.

Figure 2 illustrates the basic structure and working principle of the proposed DNA digestion/CE detection device. As shown, the device has a two-layer structure, namely, a glass-based

FIG. 1. Schematic overview of process flow in proposed microfluidic device.

layer for DNA/enzyme reaction and a PMMA-based layer for CE detection. The DNA sample is initially migrated electrophoretically through a slab-gel filled chamber in order to concentrate the DNA fragments (reservoir 1 to 2). The concentrated sample is then injected into a T-form micromixer together with the restriction enzyme (reservoirs 2 and 3 to main channel). The two species are mixed under the effects of a pinched-switching DC field and then flow downstream through a long serpentine channel. As the species flow through the channel, a reaction is induced between the DNA and the restriction enzyme by carefully controlling the channel temperature using a commercial thermo-electric (TE) cooler. The digested DNA fragments are migrated through a slab-gel filled chamber in order to separate them from the buffer and

FIG. 2. Schematic illustration of proposed two-layer microfluidic chip. (Note that the top layer (glass-based) is used for DNA/enzyme reaction and the bottom layer (PMMA-based) is used for CE detection.)

restriction enzyme (reservoir 4 to 5) and are then injected, separated, and detected in the CE channel (injection: reservoir 5 to 6, separation: reservoir 7 to 8).

III. MATERIALS AND METHODS

A. Microchannel fabrication

Figure 3 illustrates the basic steps in the fabrication process used to realize the proposed microfluidic device. In the DNA/enzyme reaction layer of the proposed device, the samples are driven electrokinetically through the mixer and serpentine channel. Thus, a silanol-group rich material is preferred as the substrate material. Consequently, the DNA/enzyme reaction layer was fabricated using low-cost microscope slides. Briefly, the microfluidic channel was patterned by coating the glass slide with a 3- μm thick AZ4620 photoresist (PR) layer, exposing the PR using UV light (Fig. 3(a)), and then etching the slide in a BOE solution for 40 min in order to obtain a channel depth of 40 μm (Fig. 3(b)). The PR layer was then stripped using a 1-M solution of KOH at a temperature of 80°C (Fig. 3(c)). Fluid via holes were drilled in a bare glass substrate (Fig. 3(d)), which was then carefully aligned with the patterned glass slide (Fig. 3(e)). The two glass slides were then sealed via a thermal fusion bonding process performed at a temperature of 580°C for 10 min (Fig. 3(f)). (Note that a full description of the glass-chip fabrication procedure is available in an earlier study by the current group.⁴⁵) In the proposed device, the digested DNA is injected and separated electrophoretically, and thus a plastic microchannel is sufficient. Consequently, the CE detection layer was fabricated on a PMMA substrate using the hot embossing process (Fig. 3(g)) and solvent bonding technique (Fig. 3(h)) developed in a previous study by the present group.⁴⁵ The glass chip and PMMA chip were finally aligned and bonded using UV glue with the PMMA CE chip on top and the glass DNA reaction chip on the bottom (Fig. 3(i)).⁴⁶ It is note that the reservoirs of the mixer were opened via the PMMA level such that sample loading and electric fields can be applied from these via-holes.

Figure 4 presents a photograph of the glass-based DNA reaction chip. (Note that the micro-mixer and serpentine channel are filled with black ink in order to better visualize their configuration.) The chip has overall dimensions of 7.6 cm $\times$ 2.6 cm, while the serpentine channel has a length of 16 cm. The various microchannels within the chip have a width and depth of 100 μm and 40 μm , respectively.

FIG. 3. Major steps in fabrication process used to realize proposed microfluidic chip.

FIG. 4. Photograph of glass-based DNA reaction chip with a width and depth of $100\ \mu\text{m}$ and $40\ \mu\text{m}$. (Note that the channel is filled with black dye for ease of visualization.)

B. Mixing experiment

The mixing performance of the T-form micromixer was evaluated experimentally using a sodium borate buffer solution containing 10^{-5} M Rhodamine B fluorescence dye and a 10 mM sodium borate running buffer ($\text{pH} = 9.2$, Showa, Japan). The two species were driven by a programmable high-voltage power supply (MP-3500, Major Science, Taiwan), and the mixing between them was observed using a fluorescence microscope equipped with a CCD module (DXC-190, Sony, Japan).

The mixing performance was evaluated by analyzing the captured images using a digital image processing technique. The color images captured by the CCD camera were converted into gray-scale images in order to obtain a better indication of the fluorescence intensity. An assumption was made that the gray-scale value of the image was directly related to the concentration level of the fluorescence dye. Hence, a gray-scale value of 1 was taken to indicate that the channel was filled with fluorescence dye, while a gray-scale value of 0 was taken to mean that the channel contained no dye. To determine the concentration distribution within the mixing channel, the gray-scale values were measured and normalized at cross-sections located at various distances downstream of the T-junction. At each cross-section, ten discrete measurements were made across the width of the channel.

The mixing ratio at each cross-section was quantified as follows:⁴⁷

$$\sigma = \left(1 - \frac{\int_0^W |C - C_\infty| dy}{\int_0^W |C_o - C_\infty| dy} \right) \times 100\%,$$

where C is the species concentration profile across the width of the mixing channel, and C_o and C_∞ are the species concentrations in the completely unmixed (0 or 1) and completely mixed states (0.5), respectively.

C. DNA digestion

The DNA digestion/CE analysis performance of the proposed microchip was evaluated using the experimental setup shown in Fig. 5. As with the mixing experiments, the sample manipulations within the chip were observed using a mercury lamp induced fluorescence

FIG. 5. Experimental setup used to evaluate performance of proposed microfluidic chip.

technique and the electrokinetic driving forces required to carry out the sample injection and separation steps were generated using a programmable high voltage power supply (MP-3500, Major Science, Taiwan). The optical signals emitted from the CE channel were detected via a PMT (Photo Multiplier Tube, R928, Hamamatsu, Tokyo, Japan) and interfaced to a PC for further processing.

Figures 6(a) and 6(b) present an exploded representation of the microfluidic chip and a summary of the experimental protocol, respectively. Since the glass chip and the PMMA chip

Step	Potential Distribution	Time Lasting
A	H2 = 25 V, H1 = 0 V	5 min
B	H2 = 1440 V, H4 = 0 V (90 V/cm)	0.125 sec
C	H3 = 1440 V, H4 = 0 V (90 V/cm)	0.125 sec
D	Repeat Step B and C TE Cooler	15 min
E	H5 = 25 V, H4 = 0 V	5 min
F	H6 = 440 V, H5 = 0 V (200 V/cm)	50 sec
G	H8 = 1000 V, H7 = 0 V (200 V/cm)	250 sec

FIG. 6. (a) Exploded representation of microfluidic device. (b) Experimental procedures and protocols for DNA digestion and CE analysis.

FIG. 7. Experimental and numerical results for flow contours within T-form micromixer given different switching frequencies with a constant electric field intensity of 90 V/cm and a 200 M Ω resistor.

were bonded together and the port 5 is the via-hole between these two chips, the digested DNA samples can be directly delivered to the CE channel. Initially, 6 μ l of ϕ X-174 DNA sample (0.45 μ g/ μ l, Takara, Japan) was placed at port 1 of the DNA digestion chip and the channel between ports 2, 3, and 4 was filled with the sodium borate buffer (10 mM, pH = 9.2). In addition, port 3 was filled with an enzyme sample comprising 1 μ l of Hae III restriction enzyme (2 units, GeneCraft, Germany), 5 μ l of buffer SH (10 $\times$, GeneCraft, Germany), and 8 μ l of sodium borate buffer. An electric field of 25 V was then applied to port 2 for 5 min to migrate the DNA sample electrophoretically through a chamber filled with viscous agarose gel (step A in Fig. 6(b)). A pinched-switching DC electric field with an intensity of 90 V/cm and a switching frequency of 4 Hz was then applied to ports 2, 3, and 4 for 15 min to mix the concentrated DNA (port 2) and digestion enzyme (port 3) and to drive the mixed solution through the serpentine DNA reaction channel to port 4 (steps B, C, and D in Fig. 6(b)). Note that the reaction channel was maintained at a constant 37°C using a TE cooler (as Fig. 6(b) step D). The digested DNA sample at port 4 was purified by applying a voltage of 25 V to port 5 for 5 min to drive the sample electrokinetically through a gel-filled chamber (step E in Fig. 6(b)). An electric field of 200 V/cm was applied at port 6 for 50 s to inject the DNA fragments into the CE analysis chip (step F in Fig. 6(b)). Finally, an electric field of 200 V/cm was applied to port 8 for 250 s in order to separate the DNA fragments within the CE channel (step G in Fig. 6(b)).

IV. RESULTS AND DISCUSSION

A. Mixing performance evaluation

Figure 7 shows the experimental and numerical results for the flow contours within the T-form micromixer for switching frequencies of 2, 4, and 7 Hz, respectively. Detailed information about the mathematical model and operating parameters is presented in Ref. [17]. Note that in every case, the pinched-switching mode is implemented using a constant electric field intensity of 90 V/cm and a 200 M Ω resistor (see Fig. 2). Since in the pinched-switching mode, the sample from the driven inlet channel partially enters the mixing channel and is then pushed back, the wavelengths of the generated waves are shorter than those produced in the conventional switching mode and have a larger amplitude. As a result, the mixing efficient is enhanced. Figure 7(a) shows that the species concentration has a wave-like distribution when the samples are injected with a switching frequency of 2 Hz. In other words, a low switching frequency of 2 Hz generates sample plugs, whose volumes are too large to be injected completely into the mixing

FIG. 8. Evolution of mixing ratio in mixing channel given different switching frequencies with a constant electric field intensity of 90 V/cm and a 200 M Ω resistor.

channel. Consequently, the two samples fail to establish intimate contact in the mixing region, and thus the mixing efficiency is reduced. As shown in Fig. 7(b), when the switching frequency is increased to 4 Hz, the contact area between the two samples in the mixing channel is increased, and thus the mixing performance is correspondingly improved. However, when the switching frequency is further increased to 7 Hz, the species flow in parallel along the channel. Consequently, the contact area between the two species is reduced, and thus the mixing performance is degraded.

Figure 8 presents the experimental and numerical results for the mixing efficiency at various cross-sections of the mixing channel for a constant electric field intensity of 90 V/cm and switching frequencies of 2, 4, and 7 Hz, respectively. At a low switching frequency of 2 Hz, the mixing efficiency has a wave-like characteristic, which indicates a periodic flow of the two

FIG. 9. Time-resolved electropherograms for digested ϕ x-174 DNA sample (Note that the injection/separation voltage is 200 V/cm.)

FIG. 10. Time-resolved electropherograms for ϕ x-174 DNA sample given digestion times of: (a) 7 min, (b) 12 min, (c) 17 min, and (d) 22 min. (Note that the injection/separation voltage is 200 V/cm.)

samples in the mixing channel. When the switching frequency is increased to 4 Hz, a mixing ratio of 97% is achieved within a distance of 1 mm from the T-junction. By contrast, the mixing efficiency at the same location given a switching frequency of 7 Hz is just 64.7%. In other words, the results confirm that the optimal mixing efficiency is obtained using a pinched-mode switching frequency of 4 Hz.

B. DNA ANALYSIS RESULTS

The DNA concentration/CE analysis performance of the proposed integrated microfluidic chip was evaluated experimentally using an Hae III digested ϕ x-174 DNA sample consisting of 11 fragments (72, 118, 194, 224, 271, 281, 310, 603, 872, 1078) and 1353 base pairs (bp). The experiments were performed using a CE buffer of 1.2% HPMC (Hydroxypropyl Methyl Cellulose) in TBE (tris-borate-EDTA) with 1% SYBR[®] green I (Molecular probes, USA)

fluorescence dye. Furthermore, the electric driving field intensity and switching frequency in the micromixer were specified as 90 V/cm and 4 Hz, respectively, while the injection/separation voltage intensity was specified as 200 V/cm. The results presented in Fig. 9 show that all 11 fragments within the sample are successfully detected within 24 min.

Figure 10 presents the CE results obtained for the ϕ x-174 DNA sample using an injection/separation voltage intensity of 200 V/cm and different DNA concentration/digestion times. Note that as in the previous example, the electric driving field and switching frequency in the micromixer were specified as 90 V/cm and 4 Hz, respectively. Furthermore, the temperature of the serpentine DNA reaction column was maintained at a constant 37 °C. As shown in Figs. 10(a) and 10(c), corresponding to DNA concentration/digestion times of 7, 12, and 17 min, respectively, the detected peaks have a poor resolution. In other words, a concentration/digestion time of 17 min is insufficient to permit the reliable CE analysis of the ϕ x-174 DNA sample. However, as shown in Fig. 10(d), given a concentration/digestion time of 22 min, all 11 peaks are clearly distinguishable within 2 min. In other words, the results confirm the ability of the proposed integrated microchip to enable the full CE analysis of the ϕ x-174 DNA sample in less than 24 min.

V. CONCLUSIONS

This paper has presented a fully electrokinetic-driven microchip for rapid DNA digestion and time-resolved CE analysis. The chip comprises two layers, namely, a glass-based layer for DNA/enzyme reaction and a PMMA-based layer for CE detection. Within the glass-based layer, the DNA and restriction enzyme are mixed electroosmotically in a T-form micromixer using a simple and stable pinched-switching DC field scheme. A success digested DNA samples were injected and separated in the PMMA-based CE channel for time-resolved capillary electrophoresis analysis of DNA digested products. It has been shown that the proposed device enables a ϕ x-174 DNA sample composed of 11 fragments to be concentrated and analyzed within 24 min. Overall, the results presented in this study provide a useful contribution to the future development of continuous bio-reaction and analysis systems.

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