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Micropreparative Fraction Collection in Microfluidic Devices

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Micropreparative fraction collection following microchip-based electrophoretic analysis of biomolecules is of major importance for a variety of biomedical applications. In this paper, we present a microfabricated device-based fraction collection system. Various size DNA fragments were separated and collected by simply redirecting the desired portions of the detected sample zones to corresponding collection wells using appropriate voltage manipulations. The efficiency of sampling and collection of the fractions was enhanced by placing a cross channel at or downstream of the detection point. Following the detection of the band of interest, the potentials were reconfigured to sampling/collection mode, so that the selected sample zone migrated to the appropriate collection well of the microdevice. The potential distribution assured that the rest of the analyte components in the separation column was retarded, stopped, or reversed, increasing in this way the spacing between the sample zone being collected and the immediately following one. By this means, a precise collection of spatially close consecutive bands could be facilitated. Once the target sample fraction reached the corresponding collection well, the potentials were switched back to separation mode. Alternation of the separation/detection and sampling/collection cycles was repeated until all required sample zones were physically isolated. The integrated device consists of a sample introduction, separation, fraction sampling, and fraction collection compartments. The feasibility of the fraction collection technique was tested on a mixture of dsDNA fragments. The amounts of DNA collected in this way were enough for further downstream sample processing, such as conventional PCR-based analysis.

While electric field-mediated separations in capillary dimensions are primarily considered as analytical tools, they can also be used in micropreparative applications. The feasibility of micropreparation was first demonstrated by Hjerten et al.,[1] who used open tubular capillaries, employing a sweep liquid at the outlet end of the capillary to move the sample components into a conventional HPLC detector and subsequently into a fraction collector. Later, porous glass junction[2] and on-column frit[3] were introduced that enabled collection without flow interruption during the electrophoresis process. In the early days of capillary gel electrophoresis (CGE), collection of small quantities of oligonucleotides was demonstrated, and the collected fractions were used in subsequent dot−blot assay.[4] Later, capillary gel electrophoresis was utilized to separate and collect multiple peaks, corresponding to denatured DNA, from mutated PCR products, and the collected fractions were reamplified by PCR and analyzed again by CGE.[5] Several other examples of capillary-based fraction collectors for DNA fragments,[6−8] peptides,[9] proteins,[10] and oligosaccharides[11] have also been reported.

Precise timing is essential for an efficient fraction collection process to avoid cross-contamination of closely migrating peaks. In most reports, a constant electric field was used; however, due to its high performance, CGE of DNA often resulted in too-narrow peak widths that represented a challenge for convenient and precise collection. Karger and co-workers[12] successfully addressed this problem by introducing electric field programming during the fraction collection process, i.e., dropping the electric field strength during the collection step. More recently, the same group developed a 12-capillary system that is capable of collecting hundreds of samples in an automated fashion requiring no interruption of the electric field during the separation/collection process.[6,11]

Microfabricated separation devices have been recently emerging due to their advantages of low reagent consumption and small sample requirement, as well as their readiness for system integration and multiplexing, consequently leading to reduction in overall processing and analysis time.[13] Microchannel networks and reservoirs are fabricated into appropriate wafer materials (glass, plastic, fused silica, etc.) using conventional techniques

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of the microelectronics industry (photolithography, etching, micromachining, molding, etc.). Separation of individual sample components is attained within the microchannels that contain appropriate separation media. Detection of the migrating components is accomplished in real time during the separation process. Parallel setup, in the way of using microchannel arrays etched into a glass wafer, enabled high-throughput processing.[14,15] In addition to separation channels, structures such as mixing compartments, reaction/incubation chambers,[16] and fraction collection units can also be fabricated on a single microdevice (system integration). Although the development of microfabricated analytical devices is rapidly progressing, the important feature of fraction collection has not yet received appropriate attention.[17]

This paper demonstrates the applicability of microchip electrophoresis for rapid and high-resolution micropreparative separation of DNA fragments using a replaceable sieving polymer medium. A monolithic microfluidic device was used for sample introduction, separation, fraction sampling, and fraction collection. Fractions of various size DNA fragments were collected for further downstream processing by conventional PCR. We envision that an array of collecting chambers and electrodes can be incorporated into a single microdevice, allowing collection of up to hundreds of fractions by simply redirecting the desired portion of the separated analyte zones by appropriate electric field manipulations. Postcollection processing and necessary analysis steps can also be integrated into the same microdevice, further increasing in this way the degree of system integration.

EXPERIMENTAL SECTION

Chemicals. TBE buffer and ethidium bromide (EthBr) were obtained from Sigma Chemical Co. (St. Louis, MO). The 100-base pair (bp) DNA ladder (Life Technologies, Gaithersburg, MD) was diluted with double deionized water (18 MΩ·cm) to the working concentrations of 5 μg/mL. Poly(vinylpyrrolidone) (PVP; MW 1 300 000) (Aldrich, Milwaukee, WI) was dissolved in 0.5× TBE buffer (45 mM Tris, 45 mM borate, 1 mM EDTA, pH 8.4), containing 0.5 μM EthBr. Generation of various size DNA fragments by PCR is described elsewhere.[18] Shortly, starting from 0.5 ng/μL pUC19 template, 101-, 187-, 299-, 427-, and 567-bp fragments were amplified, using a common forward primer and five different reversed primers (200 μM) in the reaction mixture of 1.5 mM $MgCl_2$, 250 μg/mL BSA, 20% glycerol, and 0.05 unit/μL platinum Taq polymerase.

Detection/Separation Setup. Simple cross-injector design glass microchips were used for the experiments (Micralyne, Alberta, Canada) with nominal channel dimensions of 50-μm-wide, 20-μm-deep, 8.5-cm-long separation channel (8 cm from the intersection) and 2-mm-diameter access holes. The microchannels were filled with 2% poly(vinylpirrolidone) sieving matrix in 0.5× TBE buffer, containing 0.5 μM ethidium bromide, for the size separation of double-stranded DNA fragments. An inversed

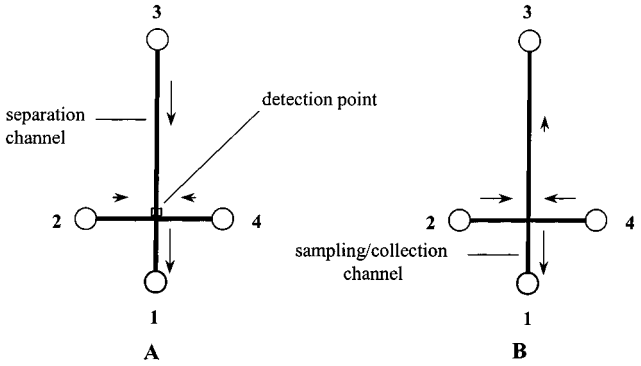

Figure 1. Schematics of fraction collection on a simple cross chip. Arrows indicate directions and relative migration velocities (not to scale) of analyte/buffer flow in the separation (A) and collection (B) modes. Circles represent fluidic reservoirs: 1, collection well; 2 and 4, side buffer reservoirs; 3, sample injection/separation buffer.

epifluorescent microscope (Eclipse TE200, Nikon, Melville, NY) was used for laser-induced fluorescent (LIF) detection with a PMT (PRI, Products for Research, Danvers, MA) for the single-point detection scheme. The 532-nm beam of a frequency-doubled Nd:YAG laser (GSF32-150, Intelite, Inc., Minden, NV) was focused in the channel with an objective (10×, 0.45 NA), and the emitted fluorescent light was collected and collimated by the same objective and passed through a dichroic beam splitter, a band-pass filter, and a spatial filter into the detector.[19] The PMT output signal was amplified by a low noise current amplifier (model SR570, Stanford Research Systems, Sunnyvale, CA), digitized using a PCI-6711 board (National Instruments, Austin, TX), and acquired by a PC for subsequent processing with Ceasar Workstation 7.0 software (CE Solutions, Mission Viejo, CA). An in-house-built four-channel, rapid ramp time, high-voltage power supply was used with gold-plated beryllium electrodes. LabView software was employed to automatically control voltage switching and data acquisition.

RESULTS AND DISCUSSION

This work reports on initial studies to prove the feasibility of high-resolution on-chip collection of multiple dsDNA fragments. The collection of sample molecules was achieved by simply redirecting the desired portions of the detected analyte bands to the corresponding collecting chambers using appropriate voltage manipulations. A simple cross microchip design was used for the proof-of-concept experiments.

Figure 1 shows the schematic representation of the microchip and the fluidics arrangement during the separation (A) and fraction collection steps (B). The DNA sample mixture was electrokinetically injected from reservoir 3 into the separation channel. First, the sample solution (2−3 μL) was placed into the reservoir followed by the application of high voltage for a short period of time (2−4 s). The voltage distribution and corresponding field strength values during the injection, separation, and collection steps are depicted in Table 1. After the injection step was accomplished, the sample was replaced by the running buffer, and the electrophoretic separation was performed in the separation channel (L = 8 cm) applying 230 V/cm electric field strength.

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Table 1. Potential Distribution for the Fraction Collection Manipulations on a Simple Cross Chip

reservoirs	1	2	3	4
channel length, mm	5	4	80	4
potentials in injection mode, V	2000	1850	0	1850
(E, V/cm[a] in the channels)	(−280)	(+24)	(+232)	(+24)
potentials in separation mode, V	2000	1850	0	1850
(E, V/cm[a])	(−280)	(+24)	(+232)	(+24)
potentials in collection mode, V	170	0	170	0
(E, V/cm[a])	(−239)	(+127)	(−15)	(+127)

[a] Positive and megative numbers for electric field strengths correspond to analyte currents directed toward and from the cross, respectively.

During the separation step, a small electric field was maintained in the side channels, to prevent the analyte diffusing into the side channels. The detection point was placed slightly upstream (100 μm) of the cross. When a band of interest was detected, the potentials were reconfigured to the collection mode, immediately following the sample zone passing the detection window (see Table 1 and Figure 1). The potentials for the collection mode for this experiment were calculated in a way to create the same electric field strength in the "sampling" channel (i.e., between the cross and reservoir 1) as it was during the separation step (between reservoir 3 and the cross). A small reversed field was maintained in the separation column to keep the rest of the DNA fragments (i.e., which were not being collected) halted or migrating slowly in the reverse direction from the cross (Figure 1B). In this way, we prevented the subsequent bands from entering the sampling channel and concomitantly contaminating the fraction being collected, especially in the case of closely migrating or poorly resolved bands. This process also increased the spacing between the target sample zone and the immediately following one. Note, that the separation channel can be held essentially field free to pause the migration during the collection step, and no apparent band broadening was observed after this step. We are currently modifying the software program to automate this voltage switch in correlation with the detected peaks to be collected. Migration of the peaks in both the separation and collection modes can be monitored by a CCD camera or by moving the detection point along the separation/sampling channel, as desired. Typically, the fractions were collected for 20 s to ensure that each analyte zone has reached the collection reservoir (5 mm from the cross). The sampling solution (~4 μL of 0.5× TBE buffer) was manually removed from the collection well after each fraction collection cycle and placed into a microfuge tube for subsequent PCR amplification.

Trace A in Figure 2 presents the profile of the 100-bp DNA sizing marker. A typical electropherogram of the five-PCR product mix, separated on-chip, is shown in Figure 2, trace B. The five PCR products were mixed and injected into the chip followed by successive collection of all the individual fragments by repeating the separation/collection cycle and appropriate voltage manipulations. Each collected fraction was used as a template for PCR reamplification of the same fragment length using the appropriate primer set. The resultant PCR products were then analyzed by microchip electrophoresis (Figure 2, traces C−G for 101-, 187-, 299-, 427-, and 567-bp fragments, respectively). Electropherograms in Figure 2 were obtained by applying the pinched injection

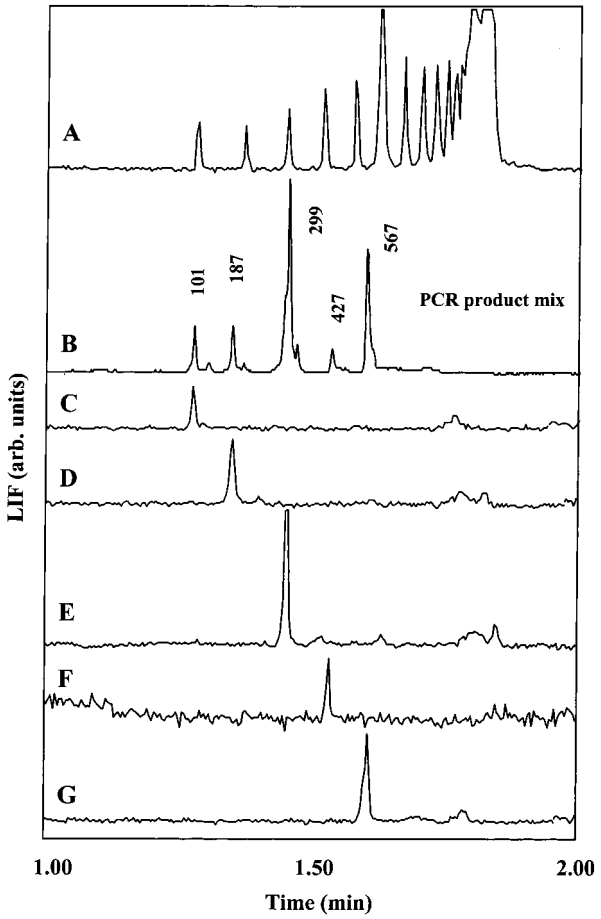

Figure 2. Microchip electropherograms: 100-bp DNA ladder (5 μg/mL) (A); five-PCR product mix, 1:10 dilution (B); and 101- (C), 187- (D), 299- (E), 427- (F), and 567-bp (G) PCR products collected successively on-chip, following a single injection of PCR product mix, and reamplified separately for the same fragment length. Conditions: 4-cm separation distance; 220 V/cm electric field strength; sieving medium 2% PVP, 0.5× TBE, 0.5 μM EthBr. "Pinched" injection was used for the sample introduction.

method.[20] These results demonstrate the successful, clean collection and subsequent amplification of all the target DNA fractions.

CONCLUSIONS AND FUTURE PROSPECTIVES

Integrated electrophoretic microfluidic devices enable precise fraction collection during electric field-mediated separation of DNA molecules and benefit in terms of miniaturization, reduced reagent consumption, speed, automation, high throughput, and performance of the analysis. Our results clearly show that micropreparative electrophoretic separation, detection, and fraction collection of DNA fragments is quite feasible and effective on the microfludic device. The amount of DNA being collected in this way, using very small subnanoliter injection volumes, proved to be enough for further downstream processing, such as conventional PCR-based analysis. Fabrication of more complex microfluidic channel networks with multiple collection wells, or having an alternative fraction collection assembly, for example, using a sampling capillary and interfacing the chip with a microtiter plate, and automation of the voltage-switching apparatus coupled to sample detection, will enable fast and precise collection of multiple

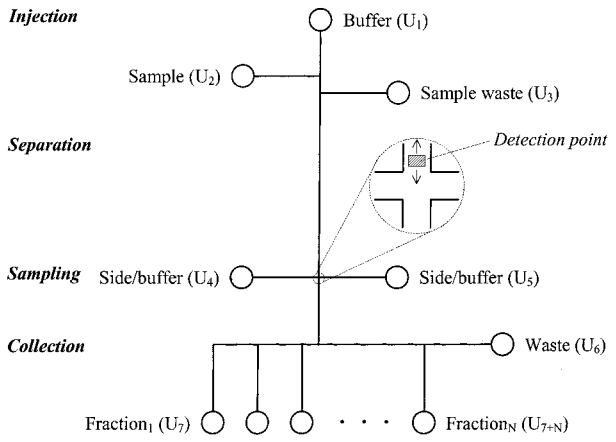

Figure 3. Schematic representation of an integrated monolithic microchip device for separation and multifraction collection.

DNA fragments (or other analytes of interest) for high-throughput applications in genomics, proteomics and metabolomics.

A schematic layout of a suggested microfabricated device with multiple collection wells is depicted in Figure 3. As described above, the efficiency of sampling and collection of the separated fractions of interest can be enhanced by placing an additional cross channel at or following the detection point in the separation channel. The integrated device consists of the injection (sample introduction), separation, fraction sampling, and fraction collection blocks (Figure 3). The fraction collection block comprises a number of collection wells and a waste reservoir. Sample introduction into the separation column can be accomplished by a simple cross[20] or double-tee injection valve.[21] The separated and detected fractions are directed and physically accumulated into the corresponding collection wells by applying appropriate potential combinations. Another possible version of the device is based on capillaries coupled to a microfluidic cross channel adaptor. The cross channels and two reservoirs on the microfabricated chip adaptor perform the fraction sampling function, essentially the same as the fraction sampling block described above. Fraction collection can be realized by interfacing the separation and collection capillaries with microtiter plates by means of robotically controlled positioning of the plates. We are currently working on the integrated capillary/microfluidic adaptor assembly for sample separation and fraction collection that will allow employing long separation distances, required for a number of applications, as well as simplify the device by using fewer fluidic reservoirs and electrical connections. In addition, automated washing/filling of the manifolds, sample pretreatment, and processing can be easily implemented in combination with conventional microplate handling methods.

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