CHIP-BASED DNA SEPARATION IN FREE SOLUTION BY INERTIAL HYDRODYNAMIC FORCES

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ABSTRACT

We report a microfluidic chip capable of carrying out separations of wide range DNA fragments in free solution by hydrodynamic separation. Hind III digested λ DNA fragments, ranging from 564 to 23,130 bp, were successfully separated in a 2 µm diameter channel by pressure-driven transport. Separations obtained in 15 minutes show the high performance of the device and the specific sizing resolution of small size DNA fragments with 2×10^5 plate number. To the authors' knowledge, this is the first report on DNA separation on a microfluidic chip without the use of sieving matrices or pulsed electric fields.

KEYWORDS: wide range DNA fragments separation, free solution, hydrodynamic flow

INTRODUCTION

DNA separations are commonly carried out by gel electrophoresis, which utilizes viscous polymer matrices and high electric fields [1]. To enhance throughput and to shorten analysis time electrophoretic separation of DNA in narrow capillaries or microchannel has been developed [2]. However, high electric fields are incompatible with the most-commonly used silicon or glass substrates, especially when the separations are performed using narrow capillaries and microchip [3]. In the last decade, there have been numerous reports of microcapillary electrophoresis (µCE) that allows high-throughput analyses by arraying multiple separations on a single device [4]. However, current µCE systems require the use of a high voltage and gel matrices for separation, and such gel-based separation has poor resolution over wide DNA size ranges [5]. Recently, several gel-free separation methods have been developed using microfabricated sieving structures to separate DNA basing on e.g. entropic trapping, liquid chromatography [6], radial migration [7], and ratchet fractionation [8]. Although suitable for separation of large DNA (>10 Kbp), these devices have limited resolution and become impractical to separate smaller DNA fragments. These methods have proven to be effective for separating DNA fragments in wide side range (from ~10 Kbp to 10 Mbp) [9]. However, estimation of DNA size in these systems is ambiguous and fabrication of nanostructured devices is increasingly complex for a high performance separation channel. Thus, it is highly desirable to develop a DNA separation microdevice that has a wide size dynamic range, high resolution and great simplicity bypassing the need for sieving matrices and pulsed electrical fields. In this paper, we present a simple and fast method for wide range DNA fragments separation in free solution microchip system by inertial hydrodynamic flow separation.

THEORY

Our device separates DNA based on wall exclusion of molecules driven by inertial hydrodynamic forces [10] as shown in Figure 1. In narrow conduits (effective size ≦ 5 µm) with a laminar flow larger molecules or particles are transported faster than smaller ones as they cannot fully access slow-flow regions near the channel walls. When two DNA molecules are transported in a pressure-driven flow inside a microchannel, the larger DNA will move faster than the smaller DNA because the larger DNA has a faster average velocity, as shown in Figure 1(b). The differential DNA mobility can be analytically estimated according to the size-dependent velocity of DNA molecules (u_p) by equation 1 [11]. On the basis of this principle, samples of DNA fragments with a wide size range have been separated in narrow capillary with resolutions comparable to gel electrophoresis in our previous report [12].

$$\bar{u}_p = \frac{2}{(R-a)^2}\int_0^{R-a} u \cdot r dr = \bar{u}(1+2\lambda-\lambda^2)$$

Where u is the average velocity of the solute, $\lambda = a/R$, a is the radius of molecule, and R is the effective radius of channel.

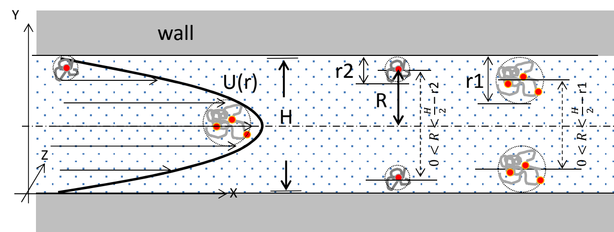

Figure 1: The principle of pressure-driving transport of DNA separation by inertial hydrodynamic force. Hydrodynamic chromatography (HDC) mechanism in fully developed pressure-driven flow microchannel.

In free solution, inertial hydrodynamic force is used for analytical separation in similar applications as traditional hydrodynamic chromatography (HDC). The separation effect occurs in packed columns or open microcapillaries and, as demonstrated here, also in flat microchannels.

EXPERIMENTAL

Hind iii digested DNA (New England Biolabs, Ipswich, MA) was labeled with PicoGreen dye (Invitrogen, Carlsbad, CA) in TE buffer at 250 ng/μL and 100 μM concentrations, respectively. All buffers were degassed and filtered through a 0.22 μm membrane filter prior to use. The mixtures were allowed to react in the dark for 1 hour at room temperature. The stained DNA stock solutions were diluted in TE buffer to a final concentration of 50-250 ng/μL dsDNA. The microchannels were formed in a 2-component PDMS from DowCorning (SYLGARD 184) with a 10:1 base to curing agent mixing ratio. The two components were thoroughly mixed and degassed in vacuum to remove bubbles, poured on the masterwafer and fully polymerized within 3 h at 75°C. A clean cover glass and the molded PDMS were oxidized for 30 sec at 40 mwatt and brought into contact immediately after oxidation. Figure 2 provides a schematic of the separation device and detection setup as well as images from actual devices, and the separation channel, which was 2×2 μm2 and 25-75 cm long. An upright fluorescence imaging microscope (BX51) equipped with a CCD camera (Intensified Retiga Fast 1394, QImaging, USA) and a computer image acquisition and processing system (Micro-Manager) was used with the prototypes to view the separations. The detection area was set at the half-length of the last channel. A time-serial fluor-image of the separated DNA fragments is shown in Figure 2(c).

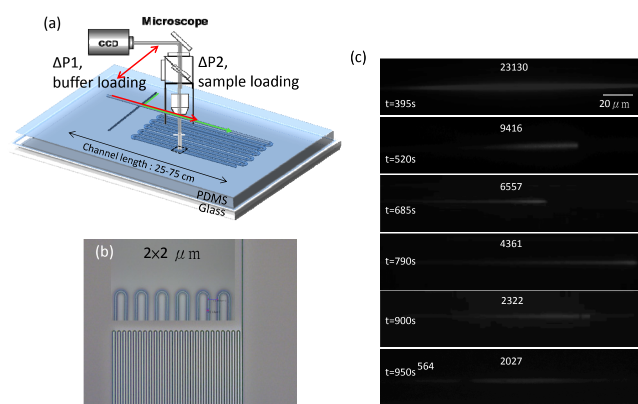

Figure 2: Schematic illustration of setup (a) and scale (b) of free solution hydrodynamic separation system on chip; (c) Time-serial fluor-image of separated DNA fragments passing the detection region.

RESULTS AND DISCUSSION

The effect of channel geometry on separation efficiency

As we know, in pressure driven free-solution hydrodynamic chromatography, where transversal concentration gradients are induced by the non-uniform flow profile (in open columns this flow profile is parabolic), the peak dispersion can be significantly reduced by reducing the (effective) diameter d of the free fluidic path. This is accomplished by using smaller beads in a packed column or using a thinner capillary as an open (i.e. non-packed) column. The selectivity in HDC is not directly influenced by this change. For fast analysis, when dispersion is dominated by the radial mass transfer, the resolution scales as

$$R \sim \sqrt{L}/d \qquad (2)$$

where L is the length of the separation column. High efficiencies can be achieved in HDC only with $d \leqq 5$μm [12].

We performed hydrodynamic separation of DNA fragments using different separation channel lengths. The results show that increasing the channel length improves resolution between the closely sized DNA fragments (2322, 2027 bp) despite the peak broadening (dispersion) that exists after long migration times in the longest length channel (Figure 3III). Reducing channel length will improve separation speed but decrease resolution (compare chromatograms of figure 3I and II). To compensate the retention times, we could either decrease the capillary length or increase the driving pressures.

The relationship of driving pressure and retention time on separation efficiency

The effect of the pressure driving force on separation resolution over the dynamic range of sample concentrations is shown in Figure 4a. Increasing the driving pressure is a simple means to increase the separation speed. However, there is an optimum pressure (75 psi) to achieve a maximum average resolution for every separation. The resolution will increase with the pressure beyond this optimum value. It has been demonstrated that separation of DNA fragments offers the best resolution for 6557 basepair DNA molecule in 2 μm by 2 μm microchannel. Bulk retention time curves for the Hind III digested λ DNA chromatograms based on the DNA length in Figure 4b. Under pressure driven flow, DNA molecules periodically stretch and tumble because the pressure-driven flow, a shear-dominant flow, includes both rota-

tional and elongational components [13]. Therefore, the DNA length scales are dynamically changing between Regition radius (Rg) and channel length (L) under channel flows. The results show that our microfluidic hydrodynamic flow separation platform is capable of wide dynamic range separation of DNA fragments.

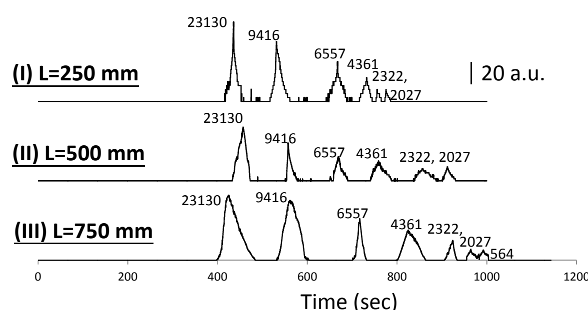

Figure 3: Hydrodynamic chromatograms of relationship between resolution and channel length for wide size range DNA separation (injection time: 60s, concentration: 250 ng/μl, separation time: 12-16 min).

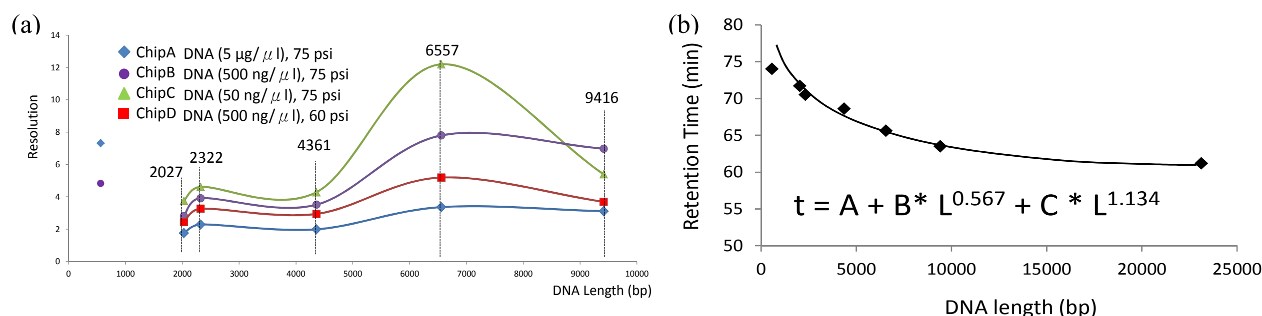

Figure 4: (a) Dynamic range of sample concentration with Pressure driving force effect. (b) Hydrodynamic chromatograms of relationship between retention time and DNA length (A= 75.18, B=-0.14, C=2.23e-4, R=0.95). The relative retention time (τ) of DNA is typically defined as the retention time of a particle divided by the retention time of an unretained solute.

CONCLUSION

We have developed a chip-based method and demonstrated its feasibility for high-resolution separations of DNA of a wide size range (from sub to tens Kbp) by inertial hydrodynamic force in free solutions. The method used a PDMS microfluidic chip, and the separations were carried out under pressure-driven flow conditions. Fluorescently labeled dsDNA molecules ranging from 564 to 23130 base pairs were separated in less than 20 min in 2x2 μm channels.

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