A COMPACT SILICON MICROPILLAR ARRAY CHIP FOR DNA CHROMATOGRAPHY: DETERMINATION OF SAMPLE SIZE AND CONCENTRATION

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ABSTRACT

This paper reports on double-stranded (ds) DNA separation by silicon micro-pillar array chips. Micro-pillars with a high aspect ratio were fabricated, allowing a large sample loading using only a small chip footprint. Our work demonstrated that a 2cm array is sufficient to separate dsDNA fragments, ranging in length from 10 base pairs (bp) to 400bp using ion-pair reversed-phase (IP-RP) chromatography. The separations were highly reproducible and a clear correlation between retention and DNA size as well as between UV absorbance signal and sample concentration was found. Subsequently, a Human Genomic polymerase chain reaction (PCR) sample was successfully analyzed by this chip.

KEYWORD

Silicon micro-pillar arrays, DNA chromatography, gradient elution mode.

INTRODUCTION

Microfabricated separation devices provide a powerful approach to miniaturization and integration of analysis systems, which have abundant applications in life science research, clinical diagnostics, drug discovery and biotechnology. Nucleic acid separation is considered as one such important application, which holds promise to enable fast and easy detection of DNA related diseases on portable systems. Liquid chromatography (LC) is a powerful technique for nucleic acid separation. Although it has been widely studied and showed many advantages in conventional high performance liquid chromatography (HPLC) columns [1], much less attention has been dedicated to nucleic acid chromatography in miniaturized systems. However, the recent development of silicon micro-pillar arrays as an alternative for the standard HPLC columns [2] brings a high interest to implement this technique into a Lab-on-a-chip system. Several important benefits of performing chromatography in micro-pillar arrays, owing to the use of advanced CMOS fabrication technology, can be listed: the ordered nature of the pillar array results in a reduction of the theoretical plate height by a factor of two compared to a packed column with equally sized particles [2], and this at a reduced pressure drop; the shape and positioning of pillars can be tailored with a high degree of freedom, allowing the conception of dedicated columns for individual applications; a small quantity of sample is required in the micro-pillar array column, which is beneficial when a minute amount of analyte is available, and also has a positive impact on the overall separation resolution and efficiency.

Among many HPLC techniques, IP-RP chromatography, along with ion-exchange chromatography (IEC), is highly suitable for DNA separations because of its excellent resolution and of the ability to separate mixtures containing a large size range of both single- and double-stranded nucleotides. Using conventional HPLC columns, the resolution of DNA fragments smaller than 1000bp is about 2-8% of their length, the analysis time ranges from less than 1 minute to 30 minutes and the reproducibility of DNA size measurement is high [1]. Moreover, it requires no prior sample preparation and it is possible to recover sample for further analysis by using volatile buffers, which can be easily removed by evaporation. In contrast to IEC, where the base pair sequence has a complex influence on retention, the elution order of dsDNA in IP-RP-HPLC reflects the size of the molecules in a more straightforward way. The retention is predominantly controlled by the overall charge and hence the number of the polynucleotides that can form ion pairs, and only marginally by the base composition. Considering these advantages of IP-RP chromatography in nucleic acid separation, we have chosen this technique to perform dsDNA separation on silicon micro-pillar array chips.

In the current paper, a short channel consisting of high aspect ratio micro-pillars was defined on silicon to perform DNA chromatography. It can separate the dsDNA fragments in a large range (10-400bp) with a minimum length difference of 5bp. The separations were highly reproducible. The dependences of the retention on the gradient steepness and organic modifier (acetonitrile) concentration were studied. Moreover, we demonstrated the separation can be used for the determination of DNA size and concentration with an example where the length and the concentration of a Human Genomic PCR product were determined.

EXPERIMENTAL

An ordered array of straight cylindrical silicon micro-pillars was fabricated using deep-UV lithography followed by a Bosch etch process [3], as shown in Figure 1. The pillar diameter is 2μm and the inter pillar distance (IPD) is 1.25μm. Thanks to a very high aspect ratio (1:25), obtained by a dedicated etching process, a large sample loading volume is possible on a small chip size. This is an important feature if the analyte has to be used for further analyses which require

a relatively large volume/concentration. As the large volume is obtained by exploiting the large pillar depth, sample overloading effects can be avoided. The channel is then sealed by anodic bonding to Pyrex glass and access holes are opened from the silicon backside. In order to prepare the reversed stationary phase, the chip was surface-functionalized to be hydrophobic by using both dodecyl-dimethylchlorosilane (C12) and hexamethyldisilazane (HMDS) coatings. A solution of 5% C12 in toluene, followed by 5% HMDS in toluene, was pumped through the chip for 12 hours.

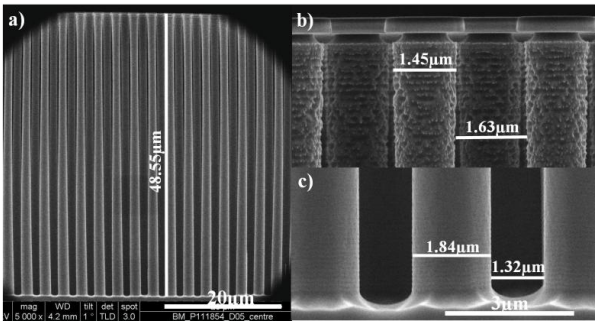

Figure 1: SEM images of high aspect ratio micro-pillar array. a) Cross-sectional view of the pillars. b) Close-up view of the top part of the pillars. c) Close-up view of the bottom part of the pillars. The height of the pillars is 48.55µm; the pillar diameter is 1.45µm at the top and 1.84µm at the bottom, corresponding to a deviation angle, with respect to the vertical, of only 0.2°.

To perform DNA separations in gradient mode, a commercial HPLC pump (RSLCnano, Dionex) was used to generate microfluids with precise mobile phase gradients and flow rates. The sample injection was controlled by two six-port valves (Achrom NV, Belgium) via a capillary (inter diameter 20µm) injection loop. A UV absorbance detector (Dionex) connected to the outlet of the chip provides the DNA UV absorbance chromatograms obtained in a 3nl UV cell at the wavelength of 254nm. All DNA separations were performed in a 2cm long by 1mm wide channel. The pressure drop is much lower and the separation time shorter, compared to longer channels [4], which are two important advantages towards integrating micro-pillar arrays into miniaturized DNA analysis systems.

RESULTS AND DISCUSSION

Four experiments for the separation of five dsDNA fragments (50-400bp) were performed, using different acetonitrile gradients in the mobile phase. In the explored range the retention capacity factors are directly proportional to the logarithm of DNA size (Figure 2a). The concentration of acetonitrile in the mobile phase needed to elute each fragment was almost identical, independent of the gradient (Table 1). This suggests that the interaction between DNA and the pillar surface is an adsorption-desorption mechanism and that the gradient, not the length of the micro-pillar array, determines the retention time. By optimizing the gradient, 10 out of 11 fragments in an ultra low DNA ladder were separated with high resolution (Figure 2b). Only the smallest fragments 10bp and 15bp were co-eluted.

Table 1: The concentration of acetonitrile in mobile phase for eluting DNA fragments in 4 different gradients (see caption in figure 2).

	Acetonitrile concentration (v/v) in %			
Size (bp)	***15min***	***30min***	***45min***	***60min***
50	9.54	9.48	9.45	9.33
100	11.03	10.96	10.98	10.88
150	12.00	11.96	12.00	11.94
250	12.84	12.85	12.92	12.86
400	13.51	13.52	13.65	13.56

By using the DNA fragments of known length as size reference, the unknown length of other DNA fragments can be determined based on its retention in the identical chromatographic profile. Moreover, also DNA concentration can be identified by the chromatogram. Figure 2c shows the chromatograms obtained by injecting a 36nl volume sample containing 4 different concentrations of 100bp DNA fragment (1.25, 2.5, 5, 10ng/µl). Within the four runs, the retention time, 13.1mins, was highly reproducible (±1.8s for the peak maximum) and the UV detected peak area showed an excellent linear dependence with the concentration of the sample (R^2=0.998) (see the inserted graph in Figure 2c). To demonstrate DNA length and concentration determination, a 135bp PCR-amplified human gene fragment was tested (Figure 2d). Firstly, five DNA fragments (50 to 400bp) were separated as size markers. Based on this, the dependence of capacity factor k on fragment length was determined: k=2.89*log(bp)-2.93, R^2=0.991. Then, in the identical condition, the human gene fragment without prior purification after PCR amplification was injected in four runs with different dilution rates in pure water (10, 20, 30 and 40 times). From the average retention value 3.22 (with standard deviation of 2.21%) of the four identical runs, the DNA length was calculated as 134.6 ±2.9bp. Using the concentration calibration curve in Figure 2c, the measured DNA concentration was converted from the peak area as 0.020 ±0.002µg/µl.

Additionally, the DNA fragment was purified since the PCR leftover reagents had less retention and thus were eluted and detected as a large peak at the void time, t_0 (see Figure 2d). This example demonstrates the high accuracy of DNA length measurement in micro-pillar array using IP-RP chromatography with simultaneous sample purification and quantification of the concentration.

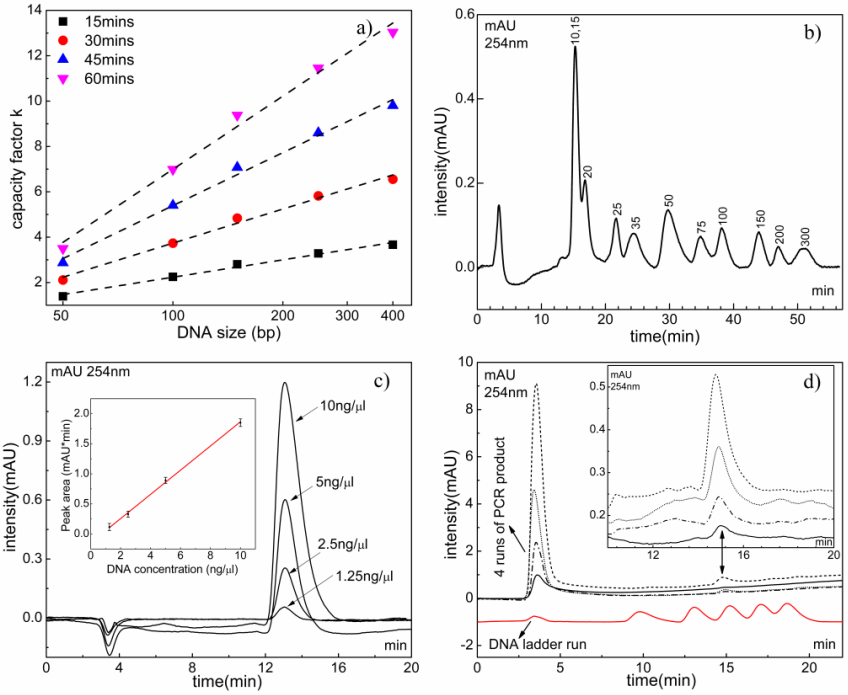

Figure 2: a) Semilogarithmic plot of capacity factors of five dsDNA fragments (36nl mixture of 50, 100, 150, 250 and 400bp dsDNA, 0.01µg/µl each) in four gradient conditions. b) A separation of an 11 fragment, ultra low range DNA ladder (36nl, 0.05µg/µl) by IP-RP chromatography. c) A calibration of UV absorbance peak area with dsDNA 100bp concentration. d) Chromatograms of five DNA fragments (36nl mixture of 50, 100, 150, 250 and 400bp, 0.01µg/µl each) (red curve) and PCR amplified human gene fragment, 135bp (black curves). In the inset, part of the chromatogram is enlarged to show the peaks corresponding to the 135bp fragment closely. Chromatographic conditions: mobile phase, 0.1M TEAA, pH 7.0, acetonitrile concentration changes a) from 8 to 15% linearly in 15, 30, 45 and 60 min; b) from 5 to 15% linearly in 60 min; c) and d) from 8 to 15% linearly in 20 min. Flow rate, 1µl/min; temperature, 25˚C; Detection, UV detector cell, 3nl, 254nm.

CONCLUSION

Silicon micro-pillar based IP-RP chromatography using gradient mode has been proven to be a promising miniaturized format for the separation of relatively short dsDNA. A short (2cm long) chip proved to be efficient for high-resolution separations. The experiments with different gradients indicated an on-off nature of the interaction between the DNA molecule and the pillar surface and thus it would be possible to further reduce the chip length and shorten the separation time without losing too much in resolution. Thanks to the high retention reproducibility and the high detection sensitivity, separations can be used for the determination of dsDNA sample length and concentration. Furthermore, PCR-amplified DNA fragments can be quickly separated from the other reaction components, identified and quantified in a single step by using IP-RP chromatography in micro-pillar arrays.

REFERENCES

[1] C.G. Huber, *Micropellicular stationary phases for high-performance liquid chromatography of double-stranded DNA*, J. Chromatogr. A, 806, **pp**.3-30 (1998).

[2] W. De Malsche, H. Eghbali, D. Clicq, J. Vangelooven, H. Gardeniers and G. Desmet, *Pressure-driven reverse-phase liquid chromatography separations in ordered nonporous pillar array columns*, Anal.Chem.**,7**, **pp**.5915-5926 (2007).

[3] W. De Malsche, L. Zhang, J. Op De Beeck, J. Vangelooven, B. Majeed and G. Desmet, *Micron-sized pillars for ion-pair reversed phase DNA chromatography*, J. Sep. Sci., 33, **pp**.3613-3618 (2010).

[4] L. Zhang, H. Gardeniers, C. Van Hoof, G. Desmet and W. De Malsche, *Silicon micropillar array chip for ion-pair reversed-phase DNA chromatography using gradient elution mode*, Proc. Micro Total Analysis Systems 2011, Seattle, WA, USA, **pp.**641-643 (2011).

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