Spiral microchannels on a CD for DNA hybridizations

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Abstract

Microfluidic devices on CD have benefited from the advantages of centrifugal pumping. To date, only channels in the radial direction have been used in these devices. In this work, we have designed numerous spiral channels on CD. Through a two-step assembly process, these spiral channels intersect with pre-immobilized radial DNA probe line to form an array of DNA that covers the whole CD surface. In our experiments, 96 radial channels were used for the first step of DNA probe immobilizations. Then, 96 spiral channels were used for the second step of hydrodynamic DNA sample hybridizations. The 96 $\times$ 96 intersections between the spiral channels and radial lines of DNA probes created a multi-sample–multi-probe array on a CD.

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1. Introduction

Currently, DNA microarray chips are constructed either by on-chip synthesis of oligonucleotide probes or by spotting of pre-synthesized probes on activated substrates [1]. Since the microfluidic method is capable of reducing the sample volume and of accelerating diffusion and hybridization, it has been reported to incorporate a pre-synthesized DNA microarray in a microfluidic chip [5]. Other groups have reported the spotting of a low-density microarray in a microfluidic channel [2–4,15,22,23]. In the creation of microarrays, some groups have used parallel straight microchannels on two separate chips consecutively so that a microarray is generated at the intersections of the two sets of microchannels [6,7]. This approach has been extended to address more sophisticated biochemical questions [8,19]. To date, this concept has been demonstrated as many as a 16 $\times$ 16 array [8], but has not been performed on arrays with higher densities, possibly because of formidable liquid handling in too many channels. To assist in microchannel liquid delivery, DNA hybridizations have been achieved on CD in which the centrifugal force has been used to pump liquids in radial channels in which a microarray is spotted [9]. So far, a methodology that combines the versatile intersection approach and the convenient centrifugal pumping has been elusive because no centrifugal pumping method can be generated in the circular channels that would intersect with the radial probe lines.

As inspired by these work, we develop a full microfluidic method, namely microfluidic microarray assembly (MMA), in which the microarray was created by the radial channels, and the application to DNA hybridization is conducted in the spiral channels. The specially design spiral channel, in which centrifugal pumping is possible, substitutes the circular channel as the sample channel to form intersections with the radial lines in order to form a DNA microarray.

The microarray assembly consists of two channel plates that are fabricated on CD-like PDMS chips and one CD-like glass wafer as the common chip used as the test chip (Fig. 1). In the first step, channel plate 1 is assembled with the common chip; whereas in the second step, after removing channel plate 1, channel plate 2 is assembled with the common chip. In both steps, liquid flows in the channels are driven by centrifugal pumping obtained by spinning the assembly. The use of centrifugal pumping for analysis on CD has been widely reported [10–14]. In our
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Fig. 1. Schematics of the MMA concept. With channel plate 1, 24 DNA probes were immobilized (solid radial lines marked as a–x). With channel plate 2, four samples (hollow spiral lines marked by 1–4) were hybridized to the 24 probe lines. Liquid flow in channel 1 was indicated by black color and the two arrows.

hands, we exploited centrifugal pumping for two times, first in the radial microchannels, and then in the spiral channels. For clarity in the schematic diagram of Fig. 1, four spiral channels are shown to intersect with the 24 radial lines to form a $24 \times 4$ array. In our work, we have constructed 96 radial channels and 96 spiral channels to realize a $96 \times 96$ array as given in Fig. 2.

When the microarray assembly is spun for the first time, the liquid (containing probes) in the radial channel flows radially outwards. When the MMA (with channel plate 2) is spun for the second time, an along-channel component of the radial centrifugal force is developed along the spiral channel. This component force results in the liquid flow (now containing DNA samples) in the spiral channels, which intersect with the line microarray created in the previous step to form a spot microarray. With the convenient liquid handling offered by this MMA method, the realization of a microarray with a higher probe density appears to be simpler than the intersection approach using straight parallel channels [6,7].

2. Experimental

2.1. Materials and equipment

Polydimethylsiloxane (PDMS) prepolymer (Sylgard 184) was obtained from Corning. The CD-like glass chips were obtained from Precision Glass & Optics. They were 4 in. in diameter with a 0.6 in. centre hole. The blue food dye was obtained from Scott-Bathgate (Vancouver, BC).

The probe sequences A and B tested in this work have been previously used to detect plant pathogens Didymella bryoniae (A = D6 or CGCCGATTGGACAAAACCTAAA) and Botrytis cinerea (B = B1 or CGCCAGAGAATACCAAAACTC) [18]. The probes were modified at the 5'-end via an amine group with a C6 linker. A' and B' are complementary sequences of A and B, respectively, and they are labeled with fluorescein at the 5'-end. All DNA sequences were obtained from Sigma-Genosys (Oakville, ON).

The stroboscope light (Monarch, Nova-Strobe DA Plus 115) was used to visualize fluid flow in the spinning CD. The confocal laser fluorescent scanner (resolution 10 μm, Typhoon 9410, Molecular Dynamics, Amersham Biosystems) was used to detect hybridizations at the intersections between DNA samples and radial probe lines.

2.2. Fabrication of channel plates and test chip

The layout design of the radial and spiral microchannel plates is created by Visual Basic. Each of the plates consists of 96 channels of width of 60 μm and the plate size is 92 mm in diameter. The design bitmap file was printed by a 3386-dpi laser printer on a plastic transparency to create the photomask. This was then used for photolithography on a photoresist/silicon dioxide-coated 4 in. Si wafer. The exposed and developed coated Si wafer is etched by buffered HF (BOE) to create positive relief structures (20-μm high) on the Si molding master. After silanizing the Si wafer for easy mold release, PDMS was casted on it and then
cured at 60 °C for 1 h, as described by a well-established procedure [16]. This produced channels with depth of 20 μm, and widths of 100 μm at the top and 60 μm at the bottom. Solution reservoirs (2 mm in diameter) were created on the channel plates by punching the PDMS layer using a flat-tip syringe needle hole puncher. Channel plate 1 (radial) and plate 2 (spiral) were to be consecutively sealed against a common glass chip. Such a microarray assembly created a microarray of 96 × 96 = 9216.

The glass test chips were chemically modified to produce aldehyde surfaces using an established 3-aminepropyltriethoxyxilane-glutaraldehyde procedure [17].

2.3. Flow visualization in the spiral channels

The flow caused by the component centrifugal force in the spiral channel during spinning was first examined using a solution containing a blue food dye. The microchannels were illuminated by a stroboscope light at the same frequency as the rotation speed. The whole operation can be seen in the video clip in Supplementary Information.

2.4. Probe immobilization

The PDMS radial microchannel plate was sealed with the glass common chip for DNA probe immobilization. Aminated DNA probes (2 μL, 100 μM) in spotting buffer (0.15 M NaCl, 0.1 M NaHCO3, pH 8.5) were applied by a pipettor to all inlet reservoirs for DNA immobilization (500 rpm for 40 min, room temperature). Then, the chip surface was reduced (NaBH4 50 mg, 95% EtOH 10 ml, PBS 30 ml, each for 15 min), and then washed successively by 0.3% SDS (5 min), water (70 °C, 5 min), and dried by N2.

2.5. Dynamic DNA hybridization

After removing channel plate 1 from the MMA, channel plate 2 was sealed with the same common CD. Cy5-labeled complementary DNA samples (A’ and B’, 0.1–2 nM) in hybridization buffer (1 × SSC, 0.015% SDS) were added by a pipettor to all inlet reservoirs. The CD assembly was spun (1800 rpm for 3 min) on the rotating platform in a temperature-controlled box for hybridization. Afterwards, the common CD was detected for specific hybridization using a fluorescent scanner. The spiral channels can be rinsed in water for 30 s and then dried before scanning. But it was found that this extra washing step might not be necessary to remove non-specific hybridization as the liquid flow in the spiral microchannel could accomplish the task, see the results shown in Fig. 5.

3. Result and discussions

3.1. The liquid movement in the spiral channels

The liquid movement in the spiral channels was first examined using the blue-dyed solutions, as shown in Fig. 3a. The inset shows how the liquid flows from inlet A to reach outlet B (this spiral channel has the same orientation as channel 4 in Fig. 1), and how the liquid flows from inlet C to reach outlet D (see also channel 1 in Fig. 1). Careful examination of liquid flow along the spiral channels did not reveal any break in the 208-mm long spiral liquid column. A liquid meniscus break would have been resulted if there had been an uneven rate of centrifugation.
gal pumping as the liquid spiralled out from the CD centre to the rim. This continuous liquid column is essential for dynamic DNA hybridization and is possibly because our special design in the spiral channel has produced a constant centrifugal force component, leading to a uniform centrifugal pumping rate.

3.2. Hydrodynamic hybridization in the spiral channels

Hydrodynamic hybridization was performed on the MMA within the spiral channel first at room temperature, see Fig. 4a. As shown in the inset, specific hybridizations can be achieved, i.e. A' binds with A, but not B; B' binds with B, but not A. The intersections between the radial probe lines (horizontally oriented) and spiral channels (vertically oriented) help to position the hybridization locations.

The microarray image as obtained from the circular CD does not conform to the usual rectangular format of microarray data. Therefore, image transformation has been performed (see Fig. 4b). Each small image of the intersection is of the same intensity as in the original circular image, except that the distance between adjacent intersections is shorter than the real distance on the CD.

A section of Fig. 4b is depicted in Fig. 5a to illustrate more details in the hydrodynamic hybridization. Here, specific hybridizations were easily observed for the sample (2 nM, 1 µL), see the right 2 lanes. When the sample concentration is 1 nM, specific hybridizations were still observed, see the bottom inset of Fig. 5a after image enhancement. The lowest concentration observed is 0.5 nM at 1 µL, attained in 3 min. of hybridization. To improve the detection limit, a greater sample volume (i.e. 10 µL) was used. In both cases, i.e. 1 µL of 1 nM or 10 µL of 0.1 nM, the mass detection limits are the same, i.e. 1 fmol. The high detection sensitivity and fast hybridization rate can be explained by the short diffusion distance and high surface area achieved in microchannels, as previously studied by other groups [15,19,20–23].

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Fig. 5. Hybridization results in the rectangular format. (a) Hybridizations at room temperature by various sample concentrations (0.1–2 nM) and volumes (1 or 10 µL) for the study of sample utilization and detection limit. The rows represent the probes (A and B) first immobilized via the radial channels (horizontal); whereas the columns represent various samples (A' and B') introduced via the spiral microchannels in the flow direction given by the hollow arrow. The bottom inset indicated the hybridization results in the boxed region but scanned at a higher detector sensitivity. (b) Hybridizations at room temperature of sample A' to probe A, but without repeated hybridizations over 22 sites of probe B. The sample concentration is 1 nM, and the sample volumes are 5 or 10 µL. (c) Hybridizations of six columns of samples obtained at five temperatures (22.5, 31.5, 36.5, 41.5 and 48.5°C). The arrangements of sample volume (1 µL) and concentration (1, 2 and 5 nM) in all blocks are the same as in the left lowermost block.
In these hydrodynamic hybridization results, it is observed that the intensities near the inlet regions of the spiral channels are higher and the intensities at latter part of the channel are lower, see the right 2 lanes in Fig. 5a. This observation could be explained by the fact that the sample (i.e. A’ in the rightmost channel) was repeatedly hybridized with the same probe (e.g. A’ to A), and hence the sample was reduced in concentration, causing a gradual reduction in fluorescent intensity of subsequent hybridizations. This effect due to sample consumption actually illustrates the high utilization of the small volume of the precious DNA sample. On the other hand, if there were no hybridizations, the DNA sample concentration would not be reduced, and the fluorescent intensity would be unchanged. This is confirmed in Fig. 5b that the rightmost lane that sample A’, after passing by 22 probes of B, still produces the same intensity with another probe A. It is noted that in the 2nd right lane of Fig. 5b, the intensities of sample B’ gradually decreased as it sequentially hybridized with the same probe B. The repeated hybridizations will not be encountered in the usual situation when different samples and probes are used.

Hydrodynamic hybridizations in microchannels have also been carried out at higher temperatures (Fig. 5c). It was noted that the non-specific hybridization obtained at 22.5 °C disappeared when higher temperatures (31.5, 36.5, 41.5 and 48.5 °C) were used. Moreover, by comparing the hybridization results of the earlier part of the spiral channel (bottom of Fig. 5c) with those of the latter part (upper of Fig. 5c), it was found that the signal (at 48.5 °C) was not reduced as the sample flowed near the outlet. We also attributed this observation to the fact that non-specific hybridization is reduced at a higher temperature, thus allowing sufficient samples to bind to the complementary probe sites.

The hydrodynamic nature in the hybridization experiments can lead to additional advantages. In conventional hybridization, non-specific DNA molecules can only be removed by an additional washing step. In the flow, any weakly bound DNA molecules will be carried away, even during the sample delivery. In addition, the flow continually brings in new DNA molecules to hybridize unbound sites and to replace those weakly bound mismatch molecules. Therefore, the dynamic hybridization process actually combines the sample delivery and washing steps. In this regard, Fig. 5 was obtained in which no washing had been performed.

4. Conclusion

A DNA microarray has been conducted with an all-microfluidic method using the intersection between the spiral sample channels and the radial probe lines. Hybridizations using PCR products will conceivably be carried out using the spiral channel microarray assembly whose characterization is the main focus of this work. In this method, numerous advantages can be envisioned, such as multi-sample capability, high hybridization rate, and high sample utilization. Other than DNA hybridizations, other surface-based reactions, such as antibody–antigen binding and enzyme–substrate reaction, can conceivably be performed on this microfluidic microarray assembly.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.snb.2007.05.038.

References


Biographies

Xing Yue (Larry) Peng received his PhD (marine chemistry) from Xiamen University, China in 1996. During 1996–1998, he was postdoctoral researcher at Department of Biology in Xiamen University and Department of Biology and Chemistry in City University of Hong Kong. He visited Department of Chemistry in Simon Fraser University in 2003–2004. He is presently associate professor in the Department of Biology in Xiamen University. His research interests are lab-on-a-chip, especially microfluidic methods for single-cell research.

Paul C.H. Li received his PhD (chemistry) in 1995 from the University of Toronto. After postdoctoral fellowship at the University of Alberta, he was assistant professor at City University of Hong Kong. He joined the Department of Chemistry Simon Fraser University in 1999, and became associate professor in 2005. He was visiting professor at the Institute of Toxicology of Johannes Gutenberg Universitat-Mainz, Germany in 2006. His research interests are microfluidic biochip for single-cell drug resistance study and microarray-based pathogen detection. He wrote a monograph entitled “Microfluidic lab-on-a-chip for Chemical and Biological Analysis and Discovery”, published in 2006 by CRC press.

Hua-Zhong Yu received his PhD (chemistry) from Peking University (Beijing, China) in 1997 then went to California Institute of Technology (Pasadena, USA) as a postdoctoral fellow. He took up an assistant professor position at Simon Fraser University (Burnaby) in 2001 after a short stay at the NRC Steacie Institute of Molecular Sciences in Ottawa, Canada. Yu was the winner of the 2004 Canadian Society of Chemistry Fred Beamish Award, and was granted an early promotion to the rank of associate professor in 2005. His current research is focused on the surface chemistry of biochips and on the development of novel electronic and diagnostic devices.

M. (Ash) Parameswaran obtained his MSc in electrical engineering and later he completed his doctoral degree in microelectronics in 1990 from the University of Alberta (Canada). He has since been with Simon Fraser University as a professor and heads an applied research group in MEMS. During the 1998 academic year, he spent his sabbatical at the Berkeley Sensor and Actuator Center, University of California, Berkeley doing research in micro photonics and display applications. He has served as a referee and consultant for agencies such as National Institute of Standards and Technology, National Institute of Health, NATO, and United Nations.

Wa Lok (Jacky) Chou received his MSc (analytical chemistry) from the Department of Chemistry at Simon Fraser University in 2007. He is a GC/MS analyst in ALS Laboratory Group (Vancouver, Canada). His research interests include ultrasonic acoustic wave detection, and microfluidic chip fabrication and applications.