An equipment-free polydimethylsiloxane microfluidic spotter for fabrication of microarrays

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This paper presents a low-cost, power-free, and easy-to-use spotter system for fabrication of microarrays. The spotter system uses embedded dispensing microchannels combined with a polydimethylsiloxane (PDMS) membrane containing regular arrays of well-defined thru-holes to produce precise, uniform DNA or protein microarrays for disease diagnosis or drug screening. Powered by pre-evacuation of its PDMS substrate, the spotter system does not require any additional components or external equipment for its operation, which can potentially allow low-cost, high-quality microarray fabrication by minimally trained individuals. Polyvinylpyrrolidone was used to modify the PDMS surface to prevent protein adsorption by the microchannels. Experimental results indicate that the PDMS spotter shows excellent printing performance for immobilizing proteins. The measured coefficient of variation (CV) of the diameter of 48 spots was 2.63% and that of the intensity within one array was 2.87%. Concentration gradient experiments revealed the superiority of the immobilization density of the PDMS spotter over the conventional pin-printing method. Overall, this low-cost, power-free, and easy-to-use spotting system provides an attractive new method to fabricate microarrays. © 2014 AIP Publishing LLC. [<http://dx.doi.org/10.1063/1.4871935>]

I. INTRODUCTION

Over the past decade, microarrays have become essential analysis tools used in modern biological and biomedical research.¹ They enable rapid, highly parallel analysis of target molecules in an unknown sample. A microarray-based analytical strategy is quicker and more convenient than serial testing for individual analytes, and it has been successfully applied to a variety of biological assays, such as sequencing by hybridization,² mutation detection,³ assessment of gene copy number,⁴ comparative genome hybridization,⁵ drug discovery,⁶ expression analysis,⁷ immunoassays,⁸ and proteomic analysis.⁹ In general, methods for microarray fabrication can be classified into three categories: contact printing, *in situ* synthesis, and non-contact inkjet printing.^{10,11} The most straightforward approach is contact printing, also known as pin spotting. This method directly impresses the probe solution on a target slide through a pin or row of pins, but has several drawbacks such as carry-over and drying problems. Another problem associated with using pin printing devices is the variation of deposition onto glass slides, especially in protein microarrays, which leads to inhomogeneous amounts of probes on the microarray. *In situ* synthesis is an alternative fabrication method that produces microarrays with extremely high density by photochemistry. However, because of the complex nature of chemical synthesis and the expense involved in production, this method is usually inflexible and expensive. The third method is non-contact printing, which uses the same technology as computer

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printers (i.e., bubble- or inkjet) to expel small droplets of probe solution onto the target slide. Although this method can solve some problems arising from the contact between printing tool and substrate, it is also limited by a number of obstacles that are difficult and time-consuming to overcome. These challenges include the optimization of a large number of parameters such as the viscosity and surface tension of probe solution, surface energy of nozzles, ejection frequency, actuation pressure and duration of jet system, and combating variable spot morphology caused by suboptimal fabrication parameters.^{12–15}

In addition, with the spread of microarray technology, there is increasing demand for the production of different specialized microarrays with a lower density of probes for use in ordinary laboratories.^{16–18} Although all of the three above technologies are used to produce commercially available microarrays, there are few methods to economically and flexibly produce small batches of custom microarrays for prototyping experiments and specialized applications. To overcome these limitations posed by the conventional technologies used to manufacture microarray, several groups have recently introduced microfluidic printing techniques for fabricating DNA or protein microarrays in a cost-effective and flexible manner, including line-printing and spot-printing.^{19–23} Line-printing techniques generally employ a set of parallel polydimethylsiloxane (PDMS) microchannels to deposit a probe line array on substrate. Compared to the probe arrays in a spot manner, the probe line arrays need an additional patterned PDMS device with microchannels oriented in orthogonal to the printed probe lines to introduce sample solutions during hybridization or immunoassay, thus increasing the complexity of microarray operation. Spot-printing techniques can selectively pattern different biomolecules (DNA or proteins) on substrates in spot array formats. However, existing spot-printing microfluidic devices are generally fabricated by assembling two patterned PDMS layers, which require accurate alignment and bonding between two patterned PDMS layers, thus increasing fabrication complexity and cost. In addition, various problems exist while making and using these devices, such as requirement of additional applied pressure for filling the narrow PDMS channels with probe solutions, air entrapment at the dead-end of “spotting” hole, and solution leakage caused by improperly applying additional pressure on reversibly bonded PDMS stencil during the printing of probes. Thus, there is still a great need for development of new microarray fabrication techniques, requiring low production cost, simplicity, flexibility, and disposability for use in common laboratories or point of care diagnostics in resource-poor settings. Here, we developed a low-cost, power-free, and easy-to-use spotter system that was extremely compact and flexible, and met the demands for fabrication of custom microarrays. The spotter chip made of PDMS exploits arrays of micro thru-holes combined with discrete dispensing microchannels to easily fabricate user-defined microarrays. In addition, powered by pre-evacuation of its PDMS substrate,^{24,25} this spotter chip does not require any external pump, connections, or tubing to dispense or spot probe solutions. Unlike contact and non-contact printing, this spotter system is insensitive to the specific fluid properties, thus reliably producing precise, uniform microarrays.

II. EXPERIMENTAL SECTION

A. Design

The spotter chip, shown schematically in Figure 1, consists of three basic elements: reservoirs, embedded microchannels, and arrays of micro thru-holes. Different biomolecule solutions can be dispensed into different reservoirs and delivered to the micro thru-holes of the spotter chip through the embedded microchannels, and then deposited on desired spot areas. The spotter chip was made of elastomeric PDMS, which is both disposable and cost-effective, and can eliminate the risk of cross-contamination between biosamples. Furthermore, the compliant nature of PDMS allows the spotter chip to conformally contact and reversibly seal to a flat substrate so that only the thru-hole-defined areas on the substrate are exposed to solutions for biomolecule patterning. After biomolecules are immobilized on the substrate and the spotter chip is peeled off, biomolecule patterns with a shape similar to the arrays of micro thru-holes remain on the substrate. In addition, this spotter chip allows self-pumping through air absorption by PDMS without connection to an external power supply. This pumping mechanism takes advantage of the

FIG. 1. Schematic diagram of the PDMS spotter. (a) The perspective view, (b) the cross-sectional view.

inherent porosity and air solubility of PDMS. By evacuating the air dissolved in the bulk PDMS, the air concentration in PDMS decreases and the energy for pumping is pre-stored in the degassed bulk PDMS. When the degassed PDMS microdevice is brought back into the atmosphere, redissolution of air into the PDMS begins immediately. If a solution droplet is loaded into the inlet port to form a closed channel-reservoir system, the redissolution of air through the microchannel walls results in a lower pressure inside the microchannel relative to the atmospheric pressure. Thus, the solution is drawn into the channels because of this pressure difference.

B. Fabrication

The spotter chip was fabricated with standard soft lithography techniques,²⁶ as outlined schematically in Figure 2. Briefly, a two-level master was prepared on a silicon wafer from a multi-layer SU-8 (Microchem, USA) process (Figure 2(a)). Then, the PDMS pre-polymer (Sylgard 184, Dow Corning, USA) was prepared by mixing resin with crosslinker in a 10:1 (w/w) ratio and poured over the master (Figure 2(b)).

FIG. 2. Schematic illustration of the process used to fabricate the PDMS spotter.

(PVA) film was placed over the pre-polymer mixture (Figure 2(c)) and an additional weight of 5 kg was positioned on the glass plate (Figure 2(d)). The PVA film, which is both mechanically robust and water soluble, allows easy transfer of the fragile molded PDMS film from the master after curing.²⁷ The sandwiched PDMS prepolymer was cured for 24 h at room temperature. After curing, the glass plate was removed by manual lift-off and the PVA-PDMS stack was peeled off from the master. Following detachment, the combined PVA-PDMS film was aligned and bonded to a pre-punched PDMS slab by plasma treatment (Figure 2(e)), and then the bonded PDMS was immersed in DI water for several minutes to dissolve the PVA film (Figure 2(f)). Finally, the excess PDMS was trimmed with a razor blade (Figure 2(g)). Typical dimensions of the device are: microchannel = $15\ \mu\text{m}$ (height) $\times$ $200\ \mu\text{m}$ (width); thru-hole = $100\ \mu\text{m}$ (height) $\times$ $200\ \mu\text{m}$ (diameter). Before use, the spotter chip was degassed under vacuum and then sealed in air-tight packaging for ready use.

C. Surface modification

To minimize nonspecific protein adsorption to the microchannel walls of the PDMS spotter, a Polyvinylpyrrolidone (PVP) coating was used to modify the surface of PDMS according to a previously published protocol.²⁸ Briefly, the PDMS spotter chip was first treated with oxygen plasma at 150 W for 30 s and then immersed in a 20% (w/v) solution of PVP K30 for 1 h. After removal from the PVP solution, the spotter chip was washed with DI water three or four times and then dried before use. The wettability of the modified surface was also evaluated by contact angle measurements (EasyDrop, Kruss, Germany).

D. Operating procedure

The basic operating principles of the PDMS spotter are outlined in Figure 3. In the printing process of the microarray, the spotter chip was first placed in a vacuum chamber and degassed at 10 kPa for 2 h. Immediately after removal from the desiccator, the degassed PDMS spotter

FIG. 3. Operating procedure of the PDMS spotter: (a) degassing of the PDMS spotter in a vacuum chamber, (b) reversible bonding of the spotter and glass slide under atmospheric conditions, (c) sample loading and dispensing after assembly, and (d) the removal of the spotter after biomolecules immobilization on the slide and the patterning of biomolecules remaining on the substrate.

was reversibly bonded to a glass slide. If necessary, the spotter/glass assembly can be clamped by spring clips to ensure a reliable seal. Subsequently, different biomolecule solutions were dispensed onto the different inlet ports of the spotter using a pipette. Because of the evacuation-redissolution pumping mechanism of PDMS bulk, the biomolecule solution was sucked into the microchannels and micro thru-holes. After all micro thru-holes were filled with biomolecule solution, the glass slide with the adhered PDMS spotter was incubated for 24 h at ambient temperature and 70%–80% humidity following the supplier's recommendation. Finally, the PDMS spotter chip was peeled off manually and the glass slide was washed to remove all unbound molecules. Different biomolecules were immobilized on the surface of the glass substrate with the same pattern as that of the arrays of micro thru-holes on the spotter chip.

E. Chemicals and materials

PVA was obtained from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). PVP K30 was obtained from J&K Scientific Ltd (Beijing, China). Food dye solution (1%, w/v) was purchased from Jiahui Fine Chemicals Co. (Shanghai, China). DMSO (dimethyl sulfoxide) was purchased from Zhanyun Chemical Co. Ltd. (Shanghai, China). Aldehyde slides were purchased from BaiO (Tianjin, China). Alexa Fluor 488-labeled Goat Anti-Mouse IgG (H+L) was purchased from Life Technologies (Carlsbad, CA, USA). Pin-printing microarrays were fabricated with ProSysTM Gantry System (Cartesian Tech, Irvine, CA, USA) for comparison. Micrographs were acquired with an inverted microscope (IX-51, Olympus, Japan) equipped with a color CCD camera (DP70, Olympus). Grayscale values of the images were acquired with image analysis software (Image pro plus 6.0, Media Cybernetics, Bethesda, MD, USA).

III. RESULTS AND DISCUSSION

A. Self-loading

To demonstrate the feasibility of the spotter chip, we first tested its capability of automatic fluid priming or loading. To allow easy visualization, eight food dyes were used as samples. Driven by the negative pressure created by the redissolution of air into PDMS bulk, the dispensed dye solution was automatically sucked into the microchannels and gradually filled all of the available space in the closed system including microchannels and thru-holes. The chip was evaluated with an inverted microscope after the loading was completed. Figure 4 shows that all of the microchannels and micro thru-holes were filled with the dye solutions. No trace of air bubbles was observed after completion of the loading process, which verifies the availability in sample self-loading and self-dispensing of this system. No fluid leakage occurred between the spotter and glass slide, which prevents cross-contamination between different sample solutions. Incidentally, the degassing of the microfluidic spotter does not need to be performed at the time of spotting because the degassed device can be stored under gas-tight conditions (such as aluminium foil packets). Using the degas-driven flow, this microfluidic spotter avoids the requirement of valves or other actuators and external equipment to fabricate microarrays.

B. Effect of PVP modification

Because of the strong hydrophobicity of PDMS, proteins tend to readily and nonspecifically bind to its surface *via* hydrophobic-hydrophobic interactions. This surface absorption problem is enhanced by the considerable increase in surface to volume ratio on the microscale, resulting in substantial sample loss and low device performance. Therefore, this protein adsorption problem needs to be overcome before the PDMS spotter chip can be used to produce protein microarrays. After activation with oxygen plasma, a PVP coating was applied to the spotter to change the surface nature of PDMS. We characterized this coating layer by measuring the contact angles of PVP-coated PDMS surfaces and comparing them with those of control surfaces. The results show that PVP treatment decreases the contact angle of PDMS from 109° to 35°, which indicates that the modified surface is highly hydrophilic and therefore could markedly suppress nonspecific protein adsorption. We also investigated the effect of nonspecific protein absorption on

FIG. 4. An image of a PDMS spotter mounted on a glass slide after self-loading of eight colors of dye solution.

spot homogeneity. In this work, a fluorescent-tagged antibody (FITC-IgG) was used to demonstrate the suppression of the nonspecific adsorption of proteins onto the modified surface and the effect of the nonspecific adsorption of PDMS on the performance of the spotter. Figure 5 shows measured fluorescent intensity profiles of the printed protein spots along the feeding channels with and without surface modification with PVP. These data were obtained from independent arrays patterned at different locations along the feeding channel. The plotted curve contained ten data points because each feeding channel was connected to ten arrays. Each data point represents the average fluorescent intensities of six points in the same array along a feeding channel. For the unmodified PDMS spotter chips, the fluorescent intensity of spots on the microarray slide decreased with increasing channel length. This distance-dependent change in fluorescent intensity occurs because the nonspecific absorption of PDMS successively decreases the concentration of protein in the priming flow along the channels. In contrast, the modified PDMS spotter produced a more consistent fluorescent intensity. These data confirm that the modification of PDMS with PVP suppresses protein adsorption and improves the performance of the spotter.

FIG. 5. Effect of PVP modification on the protein adsorption of PDMS. Left: the average local fluorescent intensity in the feeding channel is plotted against the distance to the inlet. Right: water contact angles of PDMS and PVP-PDMS. 100 $\mu\text{g/ml}$ IgG in 10 mM PBS (phosphate buffer solution) was used to test the effect of PVP modification.

FIG. 6. A 6×8 fluorescent protein array on an aldehyde slide. Microarrays printed with (a) PDMS spotter and (b) pin-printing. $100 \mu\text{g/ml}$ IgG in 40%(v/v) DMSO and 10 mM PBS buffer was used for printing microarray.

C. Spot uniformity

The morphology and uniformity of the spots printed onto the microarray substrate affect the quality of microarray results.^{29,30} Any error introduced by imperfect spotting will inevitably impair the data output. To illustrate the power and practicality of this spotter chip, we used it to print IgG onto aldehyde slides and investigated the size uniformity and homogeneity of the spots printed on the glass slide. As shown in Fig. 6, the protein microarray produced by the PDMS spotter exhibits extremely good uniformity over the whole array. The coefficient of variations (CVs) of the horizontal diameter and area of spots were used to evaluate their shape uniformity. The calculated CVs of the horizontal diameter and area obtained from 48 spots were just 2.63% and 2.97%, respectively, for the microarray produced by the PDMS spotter. These values are superior to the CVs of spots (not including the 100 pre-print spots) produced by conventional pin printing of 4.50% and 8.56% for the horizontal diameter and area, respectively. In addition, the fluorescent intensity, which represents the number of probe molecules immobilized in a spot area, was measured to evaluate inter- and intra-spot uniformities. The calculated inter-spot CV of the mean fluorescent intensities obtained from the 48 spots was just 2.87%, while the intra-spot CVs range from 1.64% to 3.14%. These low CVs for the inter- and intra-spots are comparable to those reported for high-density microarrays.^{31–33} To further demonstrate benefits of PDMS spotter, we also investigate the impact of medium properties on the printing performance of PDMS spotter. A comparative experiment between PDMS spotter and pin-printing system was performed by printing microarrays with different buffers and additives in different concentrations. As shown in Figure 7, the diameter of spots produced by the conventional pin-printing system varied from $122 \mu\text{m}$ to $180 \mu\text{m}$ as the concentration of DMSO increased from 10% to 40%. In contrast, the diameter of spots produced by PDMS spotter was nearly constant with a value of $200 \mu\text{m}$, which was almost not affected by the properties of printing buffer. We believe that this consistency of printing is a result of physical confinement by the thru-holes in the PDMS spotter chip. The thru-hole arrays serve as selective physical barriers and allow the exposed areas of the substrate to be patterned with biomolecules. Because of the physical restraint of the thru-holes, the morphology of spots printed by the PDMS spotter chip is not affected by the viscosity and surface tension of solutions and only depends on the features of the thru-holes.

D. Immobilization density

The amount of immobilized protein can directly affect the sensitivity and detection limitation of protein microarrays. Thus, it is expected that maximum immobilization of protein is achieved on a given substrate to obtain the best performance from protein microarrays. Maximum immobilization is also desired because protein probes (mostly antibodies or antigens) are very expensive and their available concentration is limited. For both performance and economy considerations, an optimal protein concentration needs to be found to produce protein microarrays. To investigate the effect of protein concentration on the deposition density of protein, aldehyde slides were printed with a series of diluted Alexa Fluor 488-conjugated goat anti-

FIG. 7. Effect of DMSO concentration on the diameter of spots printed by PDMS spotter and pin-printing system. 100 $\mu\text{g/ml}$ IgG in different printing buffer was used to investigate the effect of fluid property.

mouse IgG in the concentration range from 20 to 200 $\mu\text{g/ml}$. Fluorescence intensity as a function of printed protein concentration is shown in Figure 8. Because the immobilized protein has a fluorescent label, the amount of immobilized protein can be determined from the fluorescence intensity of spots. The microarrays produced by the PDMS spotter exhibit much better signal intensity than those fabricated by the conventional pin-printing system. It can be easily seen that the saturated fluorescence intensity for spots printed by PDMS spotter is about twice the value of that by pin-printing system. Microarray fabricated by PDMS spotter reaches the saturated intensity value of pin-printing method with a much lower sample concentration, only 10% of that required by pin-printing method. This could be explained by the difference of incubation

FIG. 8. Comparison of spot intensity for Ig-G concentrations ranging from 20 to 200 $\mu\text{g/ml}$ between the PDMS spotter and pin-printing systems. 40% (v/v) DMSO in PBS was used as printing buffer.

environment. The activity of most proteins is kept at higher level in solution than being exposed to air, even if the humidity was kept at 70%–80% and DMSO was added into printing buffer to minimize evaporation. Furthermore, the difference of protein deposition between PDMS spotter and pin-printing system could also be explained by the amount of biomolecules available for immobilization. For PDMS spotter, microwell structures which are formed by conformally contacting the thru-holes onto a flat substrate are used for dispensing the print solution. The volume of solution confined in microwell with a height of 100 μm is about 3 nl and is much greater than the probe volume spotted by pin-printing technology, which is about 1 nl. Thus, compared to the conventional pin-printing system, the PDMS spotter can provide more probes to be immobilized on the substrate. This result suggests that the PDMS spotter can economically produce microarrays with a low concentration of probe, maintaining the same probe density as those produced by conventional spotting techniques with a high concentration of probe. In addition, the entire probe immobilization process for producing microarrays with the PDMS spotter was performed under aqueous conditions, which can prevent protein denature and maintain the biological function of proteins. Such wet-patterning should be useful for producing biochemical analytical microarrays to elucidate the functions and mechanisms of various types of proteins.

IV. CONCLUSION

In summary, we have developed a novel PDMS microfluidic spotter chip that uses arrays of micro thru-holes combined with discrete dispensing microchannels to produce precise, uniform microarrays. A degas-driven flow mechanism means that this spotter system allows equipment-free production of microarrays. Users only need to conformally contact the spotter chip to a flat substrate and add the probe solutions into the loading ports with a micropipette. This spotter system is operationally simple and offers a route to reduce the cost of microarray-based assays and increase their accessibility to most common laboratories. In addition to its low cost and simplicity of use, the PDMS spotter shows advantages in the uniformity of spots and the immobilization density of probes over the conventional spotting method.

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