Selective surface treatment of micro printing pin and its performance

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Biological microarray construction relies on the sequential deposition of liquid samples, typically by contact or ink-jet printing. One drawback of contact printing is excessive solution pickup on the pin's outer surface, resulting in inefficiency. The authors combated this problem with a simple method that treats pin surfaces selectively so the outer surface becomes hydrophobic while the inner surface remains hydrophilic. Silicon-micromachined pins were utilized to evaluate the effect. The results demonstrated elimination of preprinting, greater droplet size consistency (e.g., 42 ± 5 μm vs 63 ± 13 μm), and more spots ($\sim$800 vs $\sim$300) printed per loading. Consequently, an average spot diameter, between 30 and 100 μm, can be controlled, depending on the pin design. © *2006 American Institute of Physics*. [DOI: 10.1063/1.2337882]

Microarrays are synthesized by two distinct methods today.[1] First, they can be made photolithographically to directly synthesize high density biological arrays on the substrate, a method exemplified by Affymetrix.[2] This method, however, is too costly and time consuming for small-scale productions of custom biological microarray due to the number of photolithographic steps required for production. Second, they can be made with presynthesized oligonucleotides or polymerase chain reaction products by either contact printing,[3] eject droplet printing,[4,5] or otherwise.[6] Contact printing with robotic microarrayers, as pioneered by Brown and Botstein,[7] is widely used because of its flexibility in application. For example, no prior knowledge of genome sequence is required when printing with distinct or unknown genetic materials. Also, tips and robotics are durable and inexpensive–desirable factors when performing multiplexed printing over prolonged periods. A wide variety of contact printing devices have been fabricated to produce deoxyribonucleic acid (DNA) microarrays with high density. In addition to stainless steel, other materials such as tungsten,[8,9] microfabricated steel,[10] ceramic,[11] and silicon[6,12–14] have all shown promise in delivering precise droplet deposition. A common practice for most contact printing includes "preprinting."[15] Due to the presence of excessive solution clinging to the outer surface, a pin would deposit a number of larger droplets at the beginning of the printing run. This phenomenon persists until the excessive solution is either drained out during initial printings (usually 10–20 spots) or evaporated to the environment. Preprinting not only slows the printing time but also wastes valuable samples.

Our goal is to minimize the excessive pickup of sample solution on the outer surface of the printing pin. We approach our goal by strategically defining the wettability of the surfaces during pin fabrication (hydrophobic on the outer surface of the pin and hydrophilic on the capillary inner surface). The sample liquid is to be picked up by and confined within the wetting capillary but repelled from the nonwetting outer surface. In addition to the elimination of preprinting, we further expect more consistent printing, because the nonwetting coat at the tip end would control the Laplace pressure more precisely and enable more efficient transfer of sample liquid from the pin to the substrate.

The selective coating process, illustrated in Fig. 1, was performed through sequential surface treatments. We first thoroughly cleaned the pin with an oxidizer to render the entire surface hydrophilic. Next, the interior surfaces (tip end, liquid channel, and reservoir of the pin) were shielded with photoresist to remain hydrophilic. The photoresist of positive type (SJR 5740) was wicked into the capillary space and selectively covered the interior surfaces. The exterior surfaces (top, bottom, and side of the pin), on the other hand, were left exposed to a hydrophobic coating agent. Hydrophobic coating materials have been evaluated based on the needs that it should possess a water-repelling nature, low interaction with the biological molecules, chemical resistance, as well as its durability when subject to successive washing and drying. In this study, we used hexamethyldisilazane (HMDS)

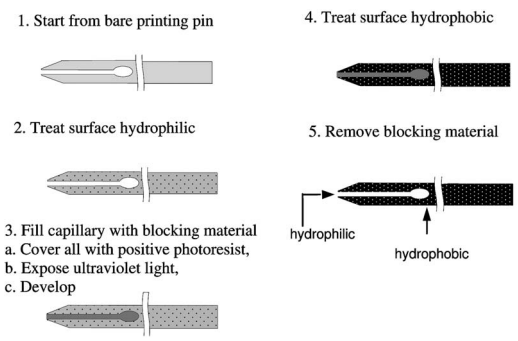

FIG. 1. Overall process to treat the pin outer surface hydrophobic and capillary inner surface hydrophilic.

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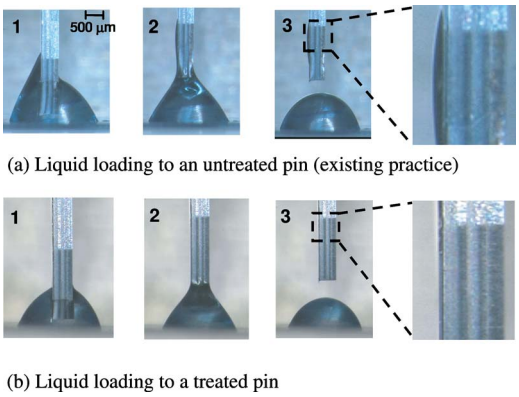

(a) Liquid loading to an untreated pin (existing practice)

(b) Liquid loading to a treated pin

FIG. 2. (Color online) Liquid loading from a sample droplet. Selective hydrophobic coating of printing pin results in better liquid loading efficiency. (a) Untreated pin picks up additional liquids at its exterior surface. (b) Treated pin shows no excess liquid pickup.

and octadecyl trichlorosilane (OTS) (water contact angle ~112°) as appropriate hydrophobic materials. Finally, the photoresist was dissolved. This dissolving step did not affect the surface wettability. In this letter, the efficacy of this method was evaluated using custom silicon-micromachined pins, whose manufacturing method and printing performance were reported elsewhere.[14] Designed to be mounted on most commercial arrayers, the silicon pins are 525 μm thick, 1400 μm wide, and 45 mm long. Note that this selective treatment can be applied to most other printing pins as well, including commercial ones.

Liquid loading experiments were performed to test the effect of selective hydrophobic treatment. A coated pin and an uncoated pin were both dipped into a 4 μl hemispherical 3XSSC droplet for 10 s and withdrawn from the droplet. The untreated pin was coated with excessive solution on its outer surface [Fig. 2(a)]. On the other hand, the treated pin was free of the excessive liquid [Fig. 2(b)]. Since the amount of liquid loading is now determined only by the volume of the capillary within the pin and not affected by how deep the pin is immersed in the sample, a consistent liquid volume is loaded regardless of the sample volume remaining in the

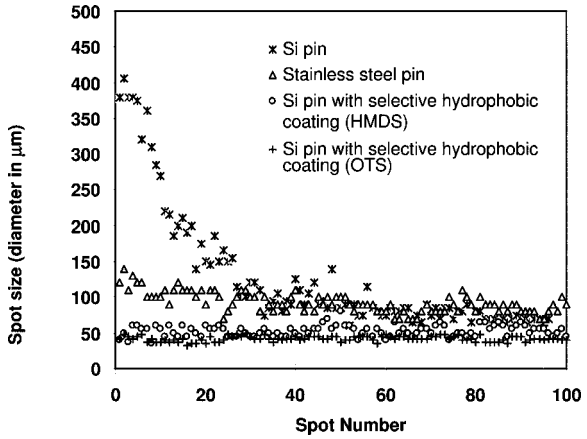

FIG. 3. Printing data with four different pins. In this experiment, a sessile drop of 5 μl oligonucleotide with 3XSSC buffer is used for loading. Both uncoated silicon pin and commercial stainless-steel pin require preprinting, as traditionally done to drain out the excess liquid on the outer surface. Selectively treated pins (by HMDS and OTS), on the other hand, exhibiting consistent spot size from the beginning of the printing process, did not need the preprinting.

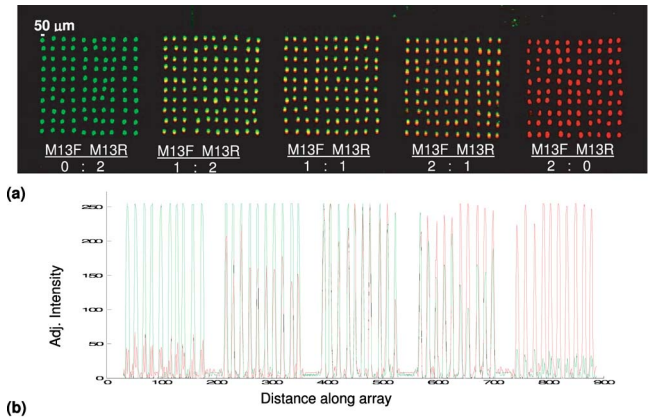

FIG. 4. (Color online) Reproducibility of spots with OTS-treated pins evaluated by two color fluorescence hybridization. (a) The two oligonucleotides, M13R and M13F, with same concentration, are mixed with ratios of 2:0, 2:1, 1:1, 1:2, and 0:2 and printed on slide. They are then hybridized with complementary oligonucleotides with Cy3 and Cy5 attached. (b) Intensity profile along a line segment. This demonstrates the spot size consistency from run to run and no sample contamination during printing.

microplate wells. This allowed for significant reduction in solution consumption.

Figure 3 shows the result of a series of printing tests on glass slides with various contact printing pins after loading from a sessile drop of 5 μl oligonucleotide with 3XSSC (saline sodium citrate) buffer. Spot diameter data were gathered by evaluating the fluorescent image of printed spots. Untreated pins, both stainless steel and silicon, printed larger spots initially and required a number of printings before spot size consistency was obtained. This is similar to typical experience with Ref. 15. Untreated silicon pins, due to their hydrophilic nature and geometry, tended to have more accumulation of excessive solution in the beginning of the run than stainless-steel pins and required approximately 30 spots run before consistent spots were reached. In comparison, treated silicon pins (both by HMDS and OTS) require little or no preprint, since the first spot printed was already small. Consistent spots of approximately 50 μm nominal diameter were achieved.

To test under typical robotic printing condition and evaluate cross contamination, another set of printing was performed with a treated silicon pin using salmon sperm DNA on polylysine slides (contact angle 65° with 3XSSC). The pins were loaded onto an Affymetrix 417 robotic arrayer. Four hundred spots were printed in one run with an average spot size of 38 μm. The two color fluorescence hybridization tests, shown in Fig. 4, were performed using complementary oligonucleotides. The two oligonucleotides, M13R and M13F, with the same concentration were mixed with ratios of 2:0, 2:1, 1:1, 1:2, and 0:2 and printed. They were then hybridized with complementary oligonucelotides with Cy3 and Cy5 attached. The spots were generated from five 10 $\times$10 spot arrays using a single pin. The data demonstrated hybridization specificity, no detectable sample carryover, precise deposition, and reproducibility of printing. Furthermore, the total number of spots created per treated pin was increased by 60% (~800 spots) relative to the approximately 300 spots per run for untreated pins. The spot diameter ranged between 40 and 60 μm, depending on the size of the treated silicon pin tip. The average spot volume was found to be ~2 pl. The robustness of the silicon pins and its hydro-

TABLE I. Overall performance of selectively treated silicon pin. In comparison with the untreated pins, the treated pins eliminate the need for preprinting (i.e., solution saving), print smaller spots at a higher precision, and print more spots per run. The data are collected using salmon sperm DNA with 3XSSC solution.

	Preprint spots	Average spot size (μm)	Standard deviation (μm)	Total spots printed
Stainless-steel pin (commercial)	10–20	100	±14	150
Si pin	~50	63	±13	300
Si pin selectively treated with HMDS	Not required	48	±8	>500
Si pin selectively treated with OTS	Not required	42	±5	>500

phobic coating were demonstrated by the long-term tests (no damage after 10 000 printing spots).

In summary, we presented a selective surface treatment to control the surface wettability of a contact printing device and evaluated its performance using silicon-micromachined pins. By tailoring the surface energy of different regions of the printing pin, we have eliminated the need for preprinting in contact printing and improved the overall consistency of the spot volumes deposited onto glass surfaces. Table I summarized the results. The selective surface treatment resulted in (1) solution saving, (2) shorter printing time, and (3) higher quality spots, which are important attributes for the creation of high throughput, larger scale microarrays.

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