Anal. Chem. **2007,** *79,* 7267−7274

Replication of DNA Microarrays Prepared by In Situ Oligonucleotide Polymerization and Mechanical Transfer

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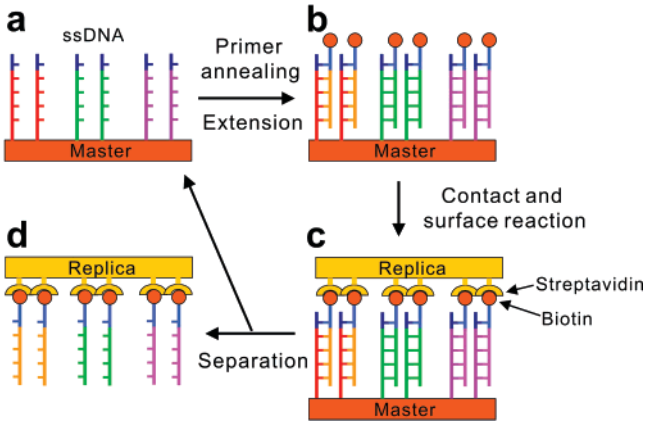
In this paper, we describe a method for replication of DNA microarrays. The approach involves in situ, enzymatic synthesis of a DNA complement array using a prefabricated master array, followed by mechanical transfer of the complement array to a second substrate. The new findings reported here include the following. DNA spots as small as ∼100 μm can be faithfully replicated, replica arrays consisting of several different oligonucleotide sequences can be prepared, and such arrays are active toward hybridization of their complements. Up to 10 replicas can be prepared from a single master with no detectable progressive degradation of their activity. DNA master arrays consisting of long DNA templates (80-mer) can be replicated, as can large-scale master arrays consisting of ∼2300 spots.

We recently reported a method for parallel replication of DNA microarrays.[1−3] The approach consists of four steps. First, a master DNA array is prepared by covalent immobilization of amine-functionalized DNA templates on an epoxy-modified glass substrate (frame a, Scheme 1). Second, biotinylated primer oligonucleotides, consisting of a single sequence, are hybridized to the distal end of the template DNA, and then the primers are extended using a T4 DNA polymerase enzyme (frame b, Scheme 1). Third, a streptavidin-coated poly(dimethylsiloxane) (PDMS) monolith is brought into contact with the master. This results in binding of the extended, biotinylated primers to the PDMS surface (frame c, Scheme 1). Fourth, the PDMS substrate is mechanically separated from the glass master array. This results in transfer of the extended primers to the PDMS surface, and it leaves the original master array ready to prepare a second replicate array (frame d, Scheme 1).

In our first report of this method,[1] we demonstrated that a single, large DNA spot (38-mer) could be replicated up to three times. Here, we expand upon the scope of this preliminary study in four ways. First, we show that master DNA microarrays consisting of three unique DNA sequences (200-μm spot size) can be faithfully replicated. Second, master arrays consisting of longer DNA sequences (80-mer) can be replicated. Third, master arrays consisting of up to ∼2300 individual 100-μm DNA spots can be replicated. Fourth, a single master can be used to prepare 10 replicate arrays without a significant decrease in the DNA-binding function of the replicas. On the basis of these findings, we concluce that mechanical replication of DNA microarrays is a robust and scaleable method for surface modification.

Scheme 1



Most existing methods for the fabrication of DNA microarrays fall into one of two categories: parallel light-directed synthesis and serial mechanical spotting of presynthesized DNA oligonucleotides.[4−8] The light-directed method combines either photolithography or micromirror[9] technology with stepwise, in situ, solid-phase oligonucleotide synthesis. Light-directed synthesis can yield spot sizes as small as 25 μm, but the length of the resulting oligonucleotides is typically limited to ∼25 bases.[8,10] DNA microarrays can also be fabricated by delivery of presynthesized oligonucleotides. Spotting is carried out using either a rigid pin to transfer solution to the array substrate or by projecting a liquid drop from a jet nozzle.

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10.1021/ac0715219 CCC: $37.00
Published on Web 09/06/2007

Table 1. Sequences of the Templates, Targets, and Primer Used in This Study[a]

name	sequence
template I	5′-5AmMC12-(iSp18)$_5$-TAT AAC AAG ACC TTC CTC AAT CCG GTG CAG AAT CGC AT-3′
template II	5′-5AmMC12-(iSp18)$_5$-CGC GGT GGA GTT CCT TCT GGC TTG GTG CAG AAT CGC AT-3′
template III	5′-5AmMC12-(iSp18)$_5$-GCC ATA CTA TCA CAA TTA CTC ATG GTG CAG AAT CGC AT-3′
long template	5′-5AmMC12-(iSp18)$_5$-GCC ATA CTA TCA CAA TTA CTC ATT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTG GTG CAG AAT CGC AT-3′
target I	5′-56FAM-TAT AAC AAG ACC TTC CTC AAT CC-3′
target II	5′-5Cy3-CGC GGT GGA GTT CCT TCT GGC TT-3′
target III	5′-5Cy5-GCC ATA CTA TCA CAA TTA CTC AT-3′
primer	5′-5BioTEG-ATG CGA TTC TGC ACC-3′

[a] Here, 5AmMC12, (iSp18)$_5$, 5BioTEG, 56FAM, 5Cy3, and 5Cy5 correspond, respectively, to an amino modifier having a 12-carbon spacer on the 5′ end of the DNA, 18-atom hexaethyleneglycol spacers repeated five times, a biotin modifier with a tetraethyleneglycol spacer on the 5′ end of the DNA, a fluorescein dye attached to the 5′ end of the DNA, a Cy3 dye attached to the 5′ end of the DNA, and a Cy5 dye attached to the 5′ end of the DNA. This is the same notation used by the DNA supplier (Integrated DNA Technologies.

This delivery method does not exert limitations on the length of the oligonucleotides, but it does require the use of presynthesized oligonucleotides and results in larger spot sizes (75−500 μm) compared to light-directed methods.[4] Additionally, the serial spotting approach can lead to an accumulation of errors, such as contamination of spots, particularly for large-scale arrays. A few other methods have been reported for fabricating microarrays, but like ours they are still in the early development stage. These include scanning probe methods[11−13] (dip-pen nanolithography, nanografting, and meniscus force nanografting) and approaches based on microcontact printing.[14]

In addition to the method shown in Scheme 1,[1] our group[2,3] and the Stellacci group[15−17] have reported related methods for parallel replication of microarrays. For example, we showed that in addition to synthesizing the template complements in situ, it is also possible to hybridize presynthesized oligonucleotides to the master array.[2] At about the same time, we described this latter method, Stellacci and co-workers reported a similar replication approach using heating,[15−17] rather than mechanical unzipping,[18,19] to separate the master and replicate oligonucleotides. They also showed that DNA features as small as 50 nm could be transferred using this general approach.[16] Finally, we recently demonstrated the use of "zip code" master arrays, which provide a means for preparing replica microarrays having different spot patterns but derived from a single master array.[3]

EXPERIMENTAL SECTION

Chemicals and Materials. Glass slides coated with an epoxy monolayer (Nexterion Slide E, Schott North America, Inc., Elmsford, NY) were used to fabricate master DNA microarrays. The PDMS monoliths were prepared from Sylgard 184 (Dow Corning, Midland, MI). Streptavidin−maleimide conjugates (Sigma S9415), (3-mercaptopropyl)trimethoxysilane (MPS) (Fluka 63800), and other chemicals for buffers or blocking solutions were obtained from Sigma-Aldrich: 20× saline−sodium citrate (SSC) buffer (Sigma S6639), 10% sodium dodecyl sulfate (SDS) solution (Sigma L4522), sodium phosphate monobasic (Sigma S0751), sodium phosphate dibasic (Sigma S0876), Triton X-100 (Sigma T8787), Trizma base (Sigma T6791), Trizma HCl (Sigma T6666), ethanolamine (Sigma E9508), 2-mercaptoethanol (Sigma M6250), and *N*-ethylmaleimide (Sigma E3876). T4 DNA polymerase (EP0061) supplied with 5× reaction buffer (335 mM TRIS-HCl pH 8.8 at 25 °C, 33 mM $MgCl_2$, 5 mM DTT, 84 mM $(NH_4)_2SO_4$), deoxyribonucleotide triphosphate (dNTP) mix (R0241), dNTP set (R0181), and nuclease-free water were used as received from Fermentas Inc. (Hanover, MD). Cy3 fluorescent dye-labeled deoxycytidine triphosphate (Cy3-dCTP) was obtained from Amersham Biosciences Corp. (Piscataway, NJ). DNA oligonucleotides were obtained from Integrated DNA Technologies (Coralville, IA). The sequences and modifications are provided in Table 1.

Characterization. A fluorescence microscope (Nikon TE2000, Nikon Co., Tokyo, Japan) equipped with appropriate filter sets (filter numbers 41001 for fluorescein, 31002 for Cy3, and 41008 for Cy5, Chroma Technology Corp., Rockingham, VT), a mercury lamp (X-Cite 120, Nikon Co), and a CCD camera (Cascade, Photometrics Ltd., Tucson, AZ), was used to acquire optical and fluorescence micrographs. Micrographs were processed using V++ Precision Digital Imaging software (Digital Optics, Auckland, New Zealand). High-density master arrays were scanned using a microarray scanner (GenePix 4000B, Molecular Devices Corp., Sunnyvale, CA).

Fabrication of Master Arrays. The master arrays were fabricated using epoxy-modified glass slides (Nexterion Slide E) as previously described[1−3] but with some modifications. Briefly, template oligonucleotide solutions (25 μM in 50 mM sodium phosphate buffer, pH 8.5) were spotted onto the glass slides using a micropipet, a manual microarrayer (Xenopore Corp., Hawthorne, NJ) in a home-built humidity chamber, or a home-built robotic microarrayer. Next, the spotted slide was placed in a chamber in which the humidity was in equilibrium with a saturated NaCl solution at 20−25 °C. After incubation, the slide was washed (at 20−25 °C) to remove unbound templates and buffer residue using

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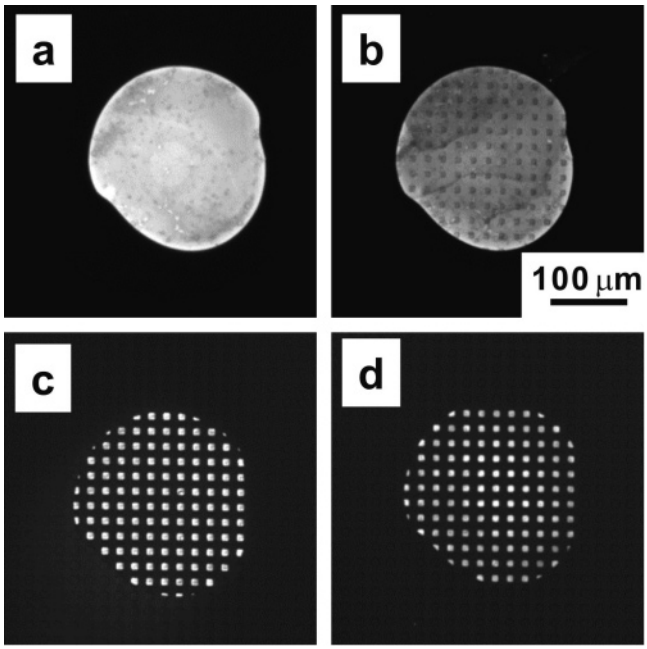
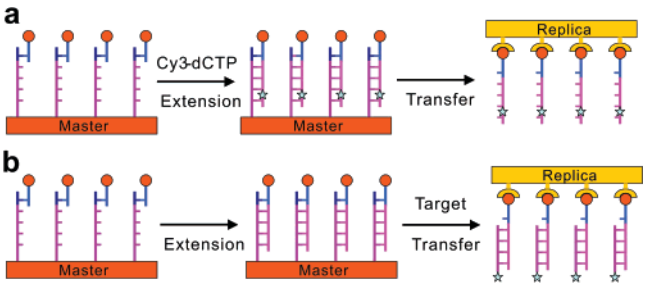


Figure 1. Fluorescence micrographs demonstrating in situ DNA primer extension on a master surface and subsequent mechanical transfer to a replica surface (Scheme 2a). (a) Fluorescence micrograph obtained from a master after a surface T4 DNA polymerase reaction incorporated Cy3-dCTP into the extended DNA primer. (b) Fluorescence micrograph obtained from the master surface after transfer of the extended primer. (c) Fluorescence micrograph obtained from the replica surface after transfer of the extended primer. (d) Same as (c), but after a third round of primer extension and transfer. The integration time was 500 ms, and the gray scales are 2000−60000 counts for (a) and (b) and 1500−10000 counts for (c) and (d).

Scheme 2



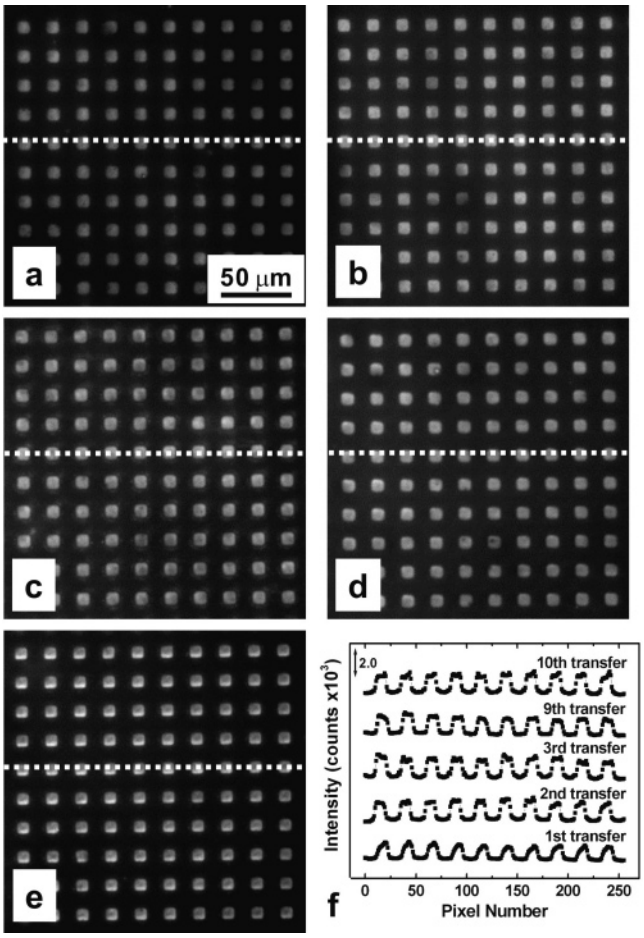


Figure 2. Micrographs demonstrating multiple replications (Scheme 2b) of a single master array incorporating a single DNA template sequence (DNA template I, Table 1) (a) Fluorescence micrograph obtained from a replica after primer extension, transfer of the polymerized DNA and hybridization of fluorescent target I (Table 1), which is complementary to the extended sequence (but not to the primer itself). (b−e) Same as (a), but after 2, 3, 9, and 10 rounds of primer extension, transfer, and hybridization, respectively. (f) Fluorescence intensity profiles obtained along the dotted white lines shown in (a−e). The integration time was 1 s, and the gray scale is 2500−5000 counts for (a−e).

the following protocol: 1 × 5 min in 0.1% Triton X-100 solution, 2 × 2 min in 1 mM HCl solution, 1 × 10 min in 100 mM KCl solution, and 1 × 1 min in Milli-Q water (18 MΩ·cm, Millipore, Bedford, MA). Next, the slide was placed in a blocking solution (50 mM ethanolamine and 0.1% SDS in 0.1 M TRIS buffer, pH 9.0) for 15 min at 50 °C. After washing with Milli-Q water for 1 min, the slide was blown dry by a N_2 stream to avoid visible drying marks on the slide surface.

Fabrication of Streptavidin-Modified PDMS Monoliths. Nanoscale, conformal contact between the master and replica surfaces is required for transfer of the replicate DNA array (Scheme 1, frame c). This requires the use of micrometer-scale canals to direct buffer solution away from the interface during contact. As previously described,[1−3] these canals were introduced into the PDMS surface using a micromolding process[20] and then the entire PDMS surface was functionalized with streptavidin. Streptavidin functionalization was carried out as follows. First, the microstructured PDMS surface was silanized with MPS. Second, a streptavidin−maleimide conjugate was covalently linked to the PDMS surface via the resulting thiol groups. The unreacted maleimide and thiol groups were blocked by incubating the functionalized PDMS in a 1.5 mM 2-mercaptoethanol solution and then in a 3 mM *N*-ethylmaleimide solution.

Replication of Master Arrays. The replication procedure used here was similar to that we reported earlier, but there were a few modifications.[1−3] First, the master slide was exposed to a primer solution, which was then extended for 5 min in a polymerase solution at 25 °C. The polymerase reaction mixture contained a T4 DNA polymerase (0.05 unit/μL) and a dNTP mixture (0.1 mM) in a polymerase reaction buffer (1×: 67 mM TRIS-HCl (pH 8.8), 6.6 mM $MgCl_2$, 1 mM DTT, 16.8 mM $(NH_4)_2SO_4$). Polymerase solutions incorporating Cy3-dCTP were prepared the same way, except using a dNTP mixture containing

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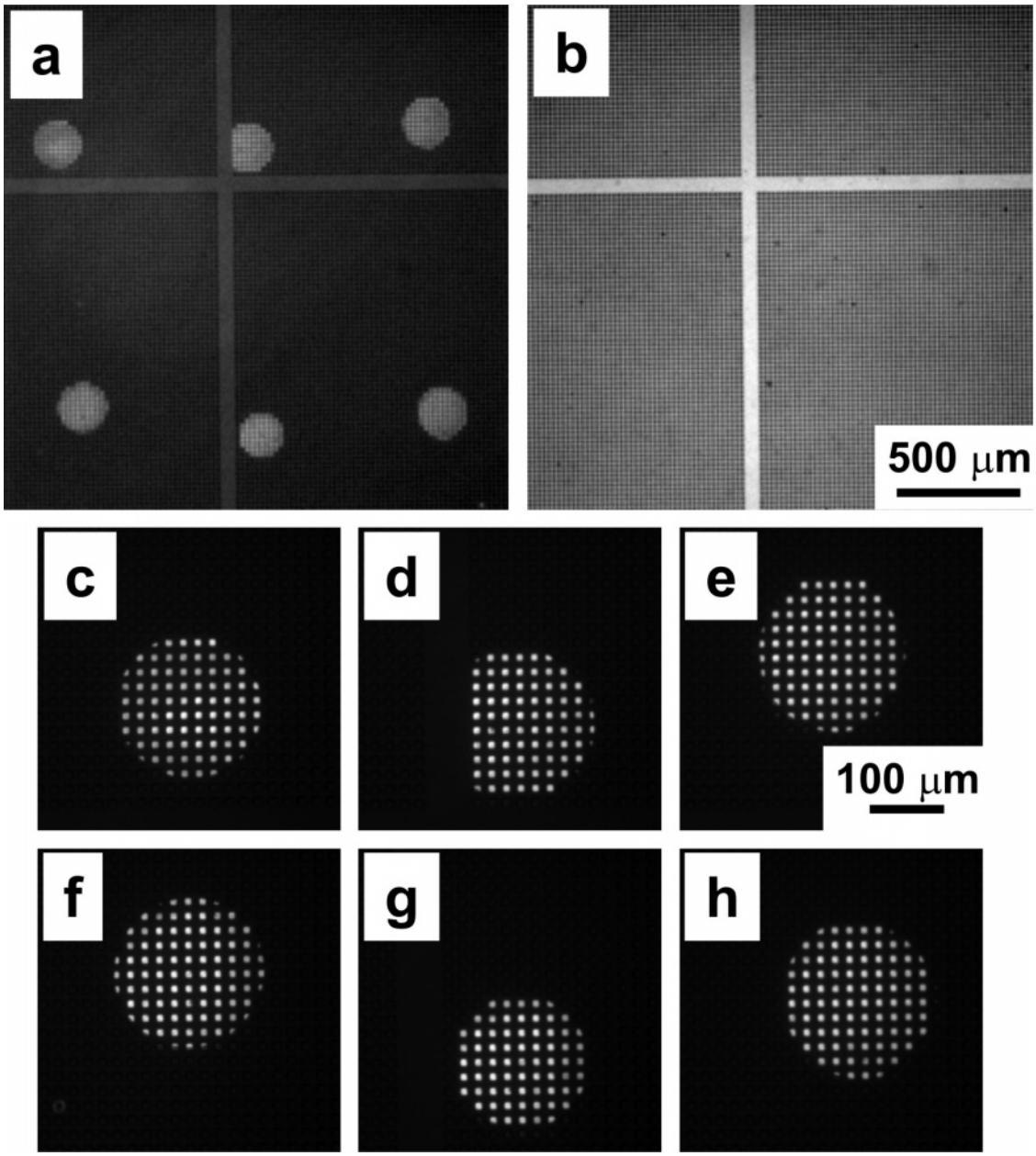

Figure 3. Micrographs demonstrating replication (Scheme 2b) of a 3 × 2 master array consisting of a single DNA template sequence (template I, Table 1). (a) Fluorescence micrograph obtained from a replica after primer extension, transfer of the polymerized DNA, and hybridization of a fluorescent target (target I, Table 1) complementary to the extended sequence (but not to the primer itself). Integration time was 1 s. The gray scale is 2100−3200 counts. (b) Optical micrograph obtained from the replica showing the drainage canal pattern. The crosslike structures are large drainage canals that are connected to the smaller canals to facilitate removal of buffer during contact of the two surfaces. (c−h) Higher magnification views of the six spots shown in (a). Registration of the spots in parts (c−h) is the same as in part a. Integration time was 1 s, and the gray scale is 3000−13000 counts.

Cy3-dCTP (0.1 mM) unless specifically mentioned otherwise. For polymerase reactions on high-density master arrays, incubation chambers (CoverWell, Grace Bio-Labs, Inc.) were used to ensure uniform spreading of the reaction mixture on the surface. Following primer extension, 4× SSC buffer (10 μL) was dropped on the master to wet the surface, and then the streptavidin-functionalized PDMS monolith was brought into contact with the surface. A pressure of 1.4 N/cm^2 was applied at 20−25 °C for 10 min. Next, the PDMS monolith was peeled off the master surface at constant separation speed (400 μm/s) using a linear motion actuator (CMA-25CC, Newport Corp., Irvine, CA), and then both surfaces were washed in buffer and blown dry.

RESULTS AND DISCUSSION

Surface Polymerization and DNA Transfer. Figure 1 demonstrates template-driven DNA polymerization on a master and subsequent transfer onto PDMS surfaces. The experiment was conducted following the approach shown in Scheme 2a. Template I solution (Table 1) was spotted onto a glass master using a manual microarrayer. This resulted in formation of a ∼200-μm-diameter template I spot. After annealing, the biotinylated primers

were extended using the polymerase reaction mixture including dye-labeled deoxycytidine triphosphate (Cy3-dCTP). The extended primers were then transferred to a PDMS replica surface as previously reported.[1−3] Figure 1a shows a fluorescence micrograph obtained from a single ~200-μm spot on the glass master after primer extension of template I and subsequent washing. The extended primer is fluorescent because of Cy3-dCTP incorporation. A control experiment confirmed that no fluorescence was detectable when the primer-annealed glass surface was exposed to the reaction mixture in the absence of the T4 DNA polymerase and washed using the same protocol used for the surface shown in Figure 1a.[1] Panels b and c in Figure 1 are fluorescence micrographs of the glass master and PDMS replica, respectively, after transfer of the extended primers. The grid pattern visible on the PDMS surface (Figure 1c) corresponds to microfabricated drainage canals (20 μm on center, 10 μm wide, and 3 μm deep), which are necessary to direct buffer solution away from the glass/PDMS interface during conformal contact.[1,3] Optical micrographs of the images shown in Figure 1c and d are provided in the Supporting Information (Figure S1a and b). The drainage canals restrict contact between the glass and PDMS surfaces to multiple square areas (10×10 μm^2) that reside between the canals. The dark areas within the spot on the master surface (Figure 1b) correspond to primer-extended DNA incorporating Cy3-dCTP that was subsequently transferred to the PDMS surface.

Figure 1d shows a PDMS replica obtained after two additional rounds of primer annealing, extension, and mechanical transfer from the same master. The average fluorescence intensities from the raised squares on the replica surfaces are 4200 ± 1100, 2200 ± 800, and 3400 ± 700 for the first, second, and third replicas, respectively, prepared from this master (fluorescence and optical micrographs of the second replica, and fluorescence intensity profiles are shown in Figure S1c−e in the Supporting Information). The fluorescence intensity profile obtained from the glass master after transfer shows that ~25% of the extended primers were transferred from the master to a replica surface (Supporting Information, Figure S1f).

Using a slightly different approach (Scheme 2b), we showed that up to 10 functional replicas can be prepared from a single master. Here, the primer extension reaction was carried out using template I in the absence of a fluorescently labeled nucleotide, and consequently, the resulting master surface is not fluorescent. However, after primer extension and transfer to the PDMS surface, the replica was exposed to fluorescently labeled DNA target I (10 μM, Table 1), which is complementary to the extended DNA sequence but not to the primer. In contrast to the approach (Scheme 2a) used to obtain the results shown in Figure 1, the experiment illustrated in Scheme 2b avoids continuous photobleaching of fluorescent dyes present on the master surface. Moreover, this approach demonstrates that the replica array is functional in that it can hybridize its complement. Figure 2a is a fluorescence micrograph obtained from a replica surface obtained after carrying out the three steps outlined in Scheme 2b. The presence of the fluorescent grid pattern indicates that extended primers transfer and are able to bind labeled DNA target I on the replica surface. Control experiments showed that there is no detectable fluorescence on the replica surface if the T4 polymerase is omitted during the primer extension step.[1] This replication cycle,

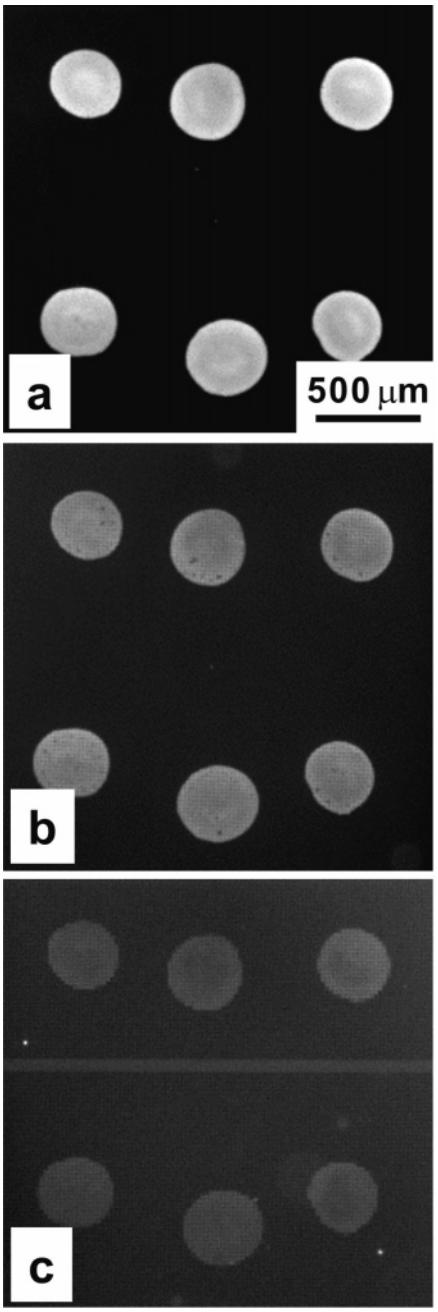

Figure 4. Micrographs demonstrating replication (Scheme 2a) of a 3×2 master array consisting of templates having long (80-mer) sequences (long template, Table 1). (a) Fluorescence micrograph obtained from a master array after a surface T4 DNA polymerase reaction incorporated Cy3-dCTP into the polymerized DNA. (b) Fluorescence micrograph obtained from the master array after transfer of the extended DNA primer. (c) Fluorescence micrograph obtained from a replica array after transfer of the extended primer. Integration time was 1 s, and the gray scales are 2500−4500 counts for (a) and (b) and 2300−3500 counts for (c).

consisting of primer extension, transfer, and hybridization with labeled DNA target I, was repeated a total 10 times using the same master. Micrographs corresponding to the second, third, ninth, and tenth cycles are presented in Figure 2b−e, respectively. The fluorescence line profiles in Figure 2f show that the contrast in fluorescence between the light and dark areas on the surfaces of these replicas were 900 ± 80, 1200 ± 90, 1210 ± 120, 1070 ± 80, and 1120 ± 100 counts, respectively. This important experiment

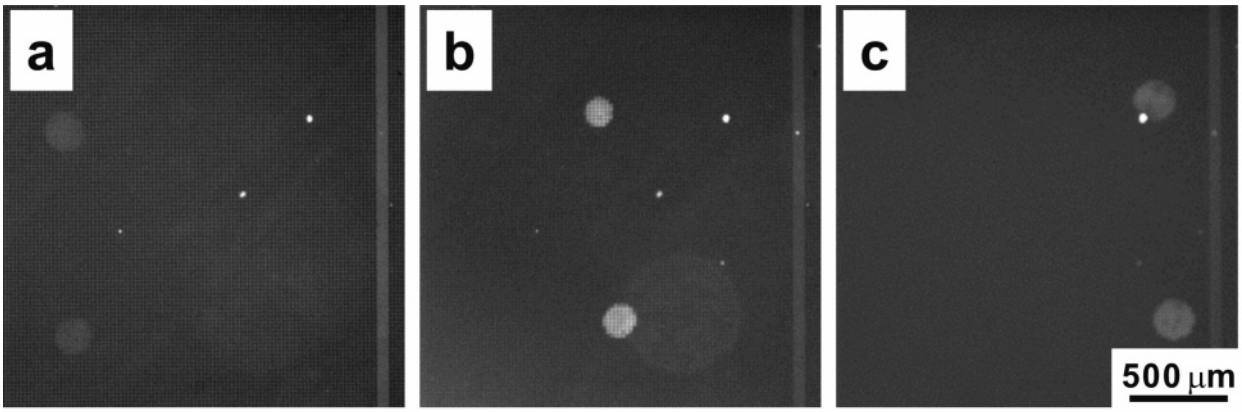

Figure 5. Fluorescence micrographs demonstrating replication (Scheme 2b) of a 3 × 2 master array consisting of three different oligonucleotide sequences (templates I, II, and III, Table 1). (a) Fluorescence micrograph obtained from the replica using a fluorescence filter for target I labeled with fluorescein. (b) Same as (a), but using a filter for target II labeled with Cy3. (c) Same as (a), but using a filter for target III labeled with Cy5. Integration time was 1 s, and the gray scales are 2400−3800 counts for (a) and (b) and 1800−3200 counts for (c).

indicates that there is no significant or progressive degradation of the functionality of the replicas up to the tenth round of replication from a single master.

Replication of a Small Master Array Having Just One Template Sequence. Thus far, our enzyme-based replication studies have resulted in transfer of a single spot. Here we show that a small array consisting of a single oligonucleotide sequence can also be transferred, and in the next section, we demonstrate that three different oligonucleotide sequences can be transferred.

The experiments corresponding to Figure 3 were performed as shown in Scheme 2b. First, a 3 × 2 master array having a single DNA template sequence (template I, Table 1) was fabricated. The primer extension reaction was carried out using unlabeled nucleotides (Scheme 2b). After transfer of the nonfluorescent extended primers, the replica was exposed to fluorescently labeled DNA target I (Table 1). Figure 3a is a fluorescence micrograph obtained from a replica surface following this series of steps. This result clearly shows that six functional spots are transferred from the master array to the replica.

The cross-like feature in Figure 1a is a large drainage canal that is fed by the smaller canals discussed earlier.[1,3] Consistent with intuition, the smaller canals always appear dark, but these large canals appear bright. Indeed, they also appear bright in the optical micrograph of the replica surface (Figure 3b). However, a series of control experiments confirmed that this is an optical effect unrelated to fluorescence (Supporting Information, Figure S2). Panels c−h in Figure 3 are higher magnification fluorescence micrographs of the six spots shown in Figure 3a. The replica spot shown in Figure 3d was truncated because of the wide drainage canal apparent in Figure 3a and b. The characteristic grid pattern arising from the smaller canals is also apparent at this magnification.

Replication of a 3 × 2 master array having a long template sequence (80-mer, long template, Table 1) was also performed using the approach shown in Scheme 2a. The sequence of this longer template was designed to incorporate a single dye-labeled nucleotide (Cy3-dCTP) exclusively at the bottom (3′ end) of the extended primer. This experiment confirms that the primer is fully extended along the length of the template and that the complete extended primer is transferred to the replica surface. Figure 4a shows six extended primer spots incorporating Cy3-dCTP on the master array. Panels b and c in Figure 4 are fluorescence micrographs of the master and a PDMS replica, respectively, after transfer of the extended primers. A more highly magnified image of each spot shown in panels b and c of Figure 4 is presented in the Supporting Information (Figure S3).The key conclusion from these experiments is that master arrays having templates consisting of up to 80 bases present in small spots can be fully replicated.

Replication of a Master Array Consisting of Multiple Template Sequences. Replication of a master array having three different template sequences was also carried out using the approach shown in Scheme 2b. First, a 3 × 2 master array having three DNA templates (left column, template I; middle column, template II; right column, template III, Table 1) was fabricated. After polymerization of DNA and transfer of the polymerized DNA, the replica PDMS surface was exposed to a mixture of fluorescent targets (targets I, II, and III; 10 μM each, Table 1) complementary to each extended sequence (but not to the primer). Three fluorescence micrographs were obtained from this single replica using a different filter set for each fluorescent target. The fluorescence micrographs shown in Figure 5 indicate that each of the three sequences present on the replica surface hybridizes with a different fluorescent target. Higher magnification images of each replica spot are presented in the Supporting Information (Figure S4). Note, however, that we also observed a very weak fluorescence signal from target II labeled with Cy3 in Figure 5a. This is because of a slight overlap of the band pass of the filter (no. 41001, Chroma Technology Corp.) used for detecting the fluorescein label on target I with the emission spectrum of the Cy3 label on target II. The important conclusion from this experiment is that the in situ DNA polymerization is correctly carried out on each template sequence and that the resulting replica spots selectively hybridize their complements.

Replication of a High-Density Master Array. Replication of a high-density master array was demonstrated using the approach shown in Scheme 2a. A master array consisting of 2304 DNA spots (template I, Table 1) was fabricated using a robotic microarrayer. After annealing the primers, a polymerase reaction mixture, including dye-labeled Cy3-dCTP, was introduced onto the master slide. Finally, the polymerized DNA incorporating Cy3-dCTP was transferred onto a replica surface. Figure 6a shows a fluorescence

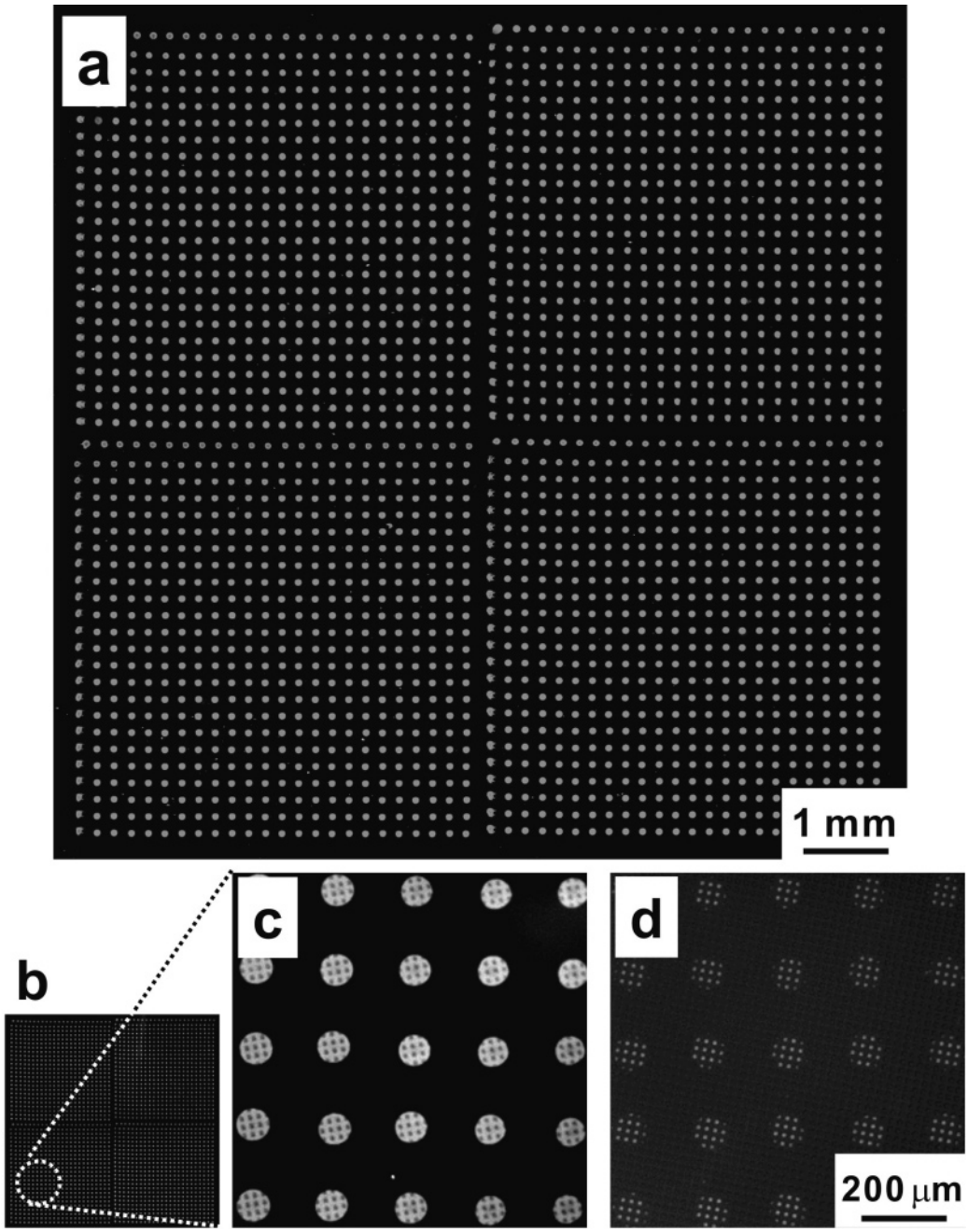

Figure 6. Fluorescence micrographs demonstrating replication (Scheme 2a) of a high-density DNA microarray. The polymerase reaction mixture included a T4 DNA polymerase (0.05 unit/μL), a dNTP mixture without dCTP (0.1 mM), and a dilute, labeled dCTP mixture (Cy3-dCTP: 10 μM, unlabeled dCTP, 90 μM) in a polymerase reaction buffer (1×: 67 mM Tris-HCl (pH 8.8), 6.6 mM $MgCl_2$, 1 mM DTT, 16.8 mM $(NH_4)_2SO_4$). (a) Fluorescence micrograph obtained by scanning the entire master (with a microarray scanner) after the surface T4 DNA polymerase reaction incorporated Cy3-dCTP into the polymerized DNA. (b) Fluorescence micrograph obtained from the master after transfer of the polymerized DNA. (c) Higher magnification view of a section of the micrograph in (b). (d) Fluorescence micrograph of the replica after transfer of the polymerized DNA. Integration time was 1 s, and the gray scales are 2600−4000 counts in (c) and 2000−4500 counts in (d).

micrograph obtained by scanning the entire master array after extending the primers and rinsing the surface. The fluorescence from the DNA spots on the master array is qualitatively homogeneous, indicating that the polymerase reaction on the high-density master is uniform. Figure 6b is a fluorescence micrograph of the entire master obtained after transfer. Panels c and d in Figure 6 are expanded views of the indicated section of the master and the corresponding replica after transfer. Figure 6c shows that a dark grid pattern is superimposed on each DNA spot, which confirms transfer of the polymerized DNA from the master array to the replica. These results indicate that this method results in faithful replication of quite large DNA microarrays.

CONCLUSION

Here, we have provided new information about several important aspects of our recently reported method for in situ synthesis and subsequent mechanical transfer of DNA to replica surfaces. The results expand the scope of this microarray replication technique, and they clarify several key questions that were not addressed in our earlier preliminary report of this method.[1]

Specifically, we have shown that DNA spots as small as ~100 μm can be faithfully replicated, that replica arrays consisting of several different oligonucleotide sequences can be prepared, that such arrays are active toward hybridization of their complements, and that up to 10 replicas can be prepared from a single master with no significant progressive degradation of their activity. Moreover, DNA master arrays consisting of long DNA templates (80-mer) could also be replicated, as could master arrays consisting of ~2300 spots. Forthcoming reports will focus on quantitative measurements of transfer efficiency and replication of arrays consisting of other biological materials (proteins, RNA, and cells).

ACKNOWLEDGMENT

Financial support for this work was provided by the U.S. Department of Energy, Office of Basic Energy Sciences (Contract DE-FG02-01ER15247). We acknowledge the Robert A. Welch Foundation and SPRING for support of the lithography facilities used to carry out this project. We also acknowledge the Microarray Core Facility (The University of Texas at Austin) and Dr. Eun-Jeong Cho for assistance with the high-density array fabrication. We thank Prof. Andrew Ellington (The University of Texas at Austin), Dr. Li Sun (Pine Research Instrumentation), Dr. Haohao Lin (3M Austin Center), and Dr. William M. Lackowski (The University of Texas at Austin) for enlightening discussions. Finally, J.K. acknowledges a Summer Graduate Fellowship from the American Chemical Society—Division of Analytical Chemistry (ACS-DAC) sponsored by Johnson & Johnson Pharmaceutical Research & Development.

SUPPORTING INFORMATION AVAILABLE

Optical and fluorescence micrographs and fluorescence intensity profiles of PDMS replicas, several control experiments, and higher magnification views of replica spots. This material is available free of charge via the Internet at http://pubs.acs.org.

Received for review July 18, 2007. Accepted August 10, 2007.

AC0715219