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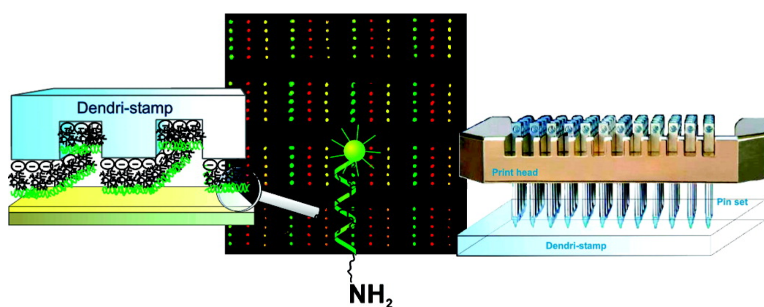

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Dendrimer-Mediated Transfer Printing of DNA and RNA Microarrays

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Abstract: This paper describes a new method to replicate DNA and RNA microarrays. The technique, which facilitates positioning of DNA and RNA with submicron edge resolution by microcontact printing (μCP), is based on the modification of poly(dimethylsiloxane) (PDMS) stamps with dendrimers ("dendri-stamps"). The modification of PDMS stamps with generation 5 poly(propylene imine) dendrimers (G5-PPI) gives a high density of positive charge on the stamp surface that can attract negatively charged oligonucleotides in a "layer-by-layer" arrangement. DNA as well as RNA is transfer printed from the stamp to a target surface. Imine chemistry is applied to immobilize amino-modified DNA and RNA molecules to an aldehyde-terminated substrate. The labile imine bond is reduced to a stable secondary amine bond, forming a robust connection between the polynucleotide strand and the solid support. Microcontact printed oligonucleotides are distributed homogeneously within the patterned area and available for hybridization. By using a robotic spotting system, an array of hundreds of oligonucleotide spots is deposited on the surface of a flat, dendrimer-modified stamp that is subsequently used for repeated replication of the entire microarray by microcontact printing. The printed microarrays are characterized by homogeneous probe density and regular spot morphology.

Introduction

DNA microarrays have rapidly developed into a fundamental tool for high-throughput genetic analysis. "DNA chips" are useful for large-scale parallel analyses of genome sequences and gene expression,[1] for the evaluation of the clinical course of tumors,[2] for detection of viruses and other pathogens,[3] for monitoring mRNA expression,[4] and for classification of human tumors.[5] Immobilization of oligonucleotides on solid surfaces is central to the design, fabrication, and operation of DNA-based microarrays.[6−9] In general, there are two methods for DNA microarray fabrication: (1) immobilization of synthetic oligonucleotides or DNA onto solid supports[6−9] and (2) direct synthesis of DNA on a chip (mostly up to 25 nucleotides).[10,11] The immobilization of DNA can be carried out by automated spotting conducted by contact printing or noncontact printing. Contact printing employs robotic systems equipped with a set of pins for dispensing pico- or nanoliters of DNA solution, where the surface is contacted for probe deposition. Noncontact printing ("ink jetting") relies on depositing the spotting solution from an array of, e.g., piezo-electrically driven pipettes onto the solid support without direct contact with the surface. DNA can be immobilized covalently by reaction between DNA modified with a reactive group (e.g., an amine) and a reactive solid support (exposing, e.g., aldehyde or epoxide groups).[6−9,12] Alternatively, DNA can be immobilized noncovalently by the adsorption on positively charged surfaces, or using Van der Waals, or hydrophobic interaction, or other.[6−9] Surfaces modified with self-assembled monolayers (SAMs),[12] polylysine,[13] polyacrylamide gel,[14] dendrimers,[15−18] or agarose films[19] were

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(1) Noordewier, M. O.; Warren, P. V. *Trends Biotechnol.* **2001**, *19*, 412−415.
(2) Golub, T. R.; Slonim, D. K.; Tamayo, P.; Huard, C.; Gaasenbeek, M.; Mesirov, J. P.; Coller, H.; Loh, M. L.; Downing, J. R.; Caligiuri, M. A.; Bloomfield, C. D.; Lander, E. S. *Science* **1999**, *286*, 531.
(3) (a) Li, J. P.; Chen, S.; Evans, D. H. *J. Clin. Microbiol.* **2001**, *39*, 696. (b) Martell, M.; Briones, C.; de Vicente, A.; Piron, M.; Esteban, J. I.; Esteban, R.; Guardia, J.; Gómez, J. *Nucleic Acids Res.* **2004**, *32*, e90.
(4) Yue, H.; Eastman, P. C.; Wang, B. B.; Minor, J.; Doctolero, M. H.; Nuttall, R. L.; Stack, R.; Becker, J. W.; Montgomery, J. R.; Vainer, M.; Johnston, R. *Nucleic Acids Res.* **2001**, *29*, e41.
(5) Perou, C. M. *Nature* **2000**, *406*, 747−752.
(6) Heise, C.; Bier, F. F. *Top Curr. Chem.* **2006**, *261*, 1−25.
(7) Del Campo, A.; Bruce, I. J. *Top Curr. Chem.* **2005**, *260*, 77−111.
(8) Pirrung, M. C. *Angew. Chem., Int. Ed.* **2002**, *41*, 1276−1289.
(9) Cheung, V. G.; Morley, M.; Auilar, F.; Kucherlapati, R.; Childs, G. *Nat. Genet.* **1999**, *21*, 15−19.
(10) Fodor, S. P.; Read, J. L.; Pirrung, M. C.; Stryer, L.; Lu, A. Y.; Solas, D. *Science* **1991**, *251*, 767.
(11) Mcgall, G. H.; Barone, A. D.; Diggelmann, M.; Fodor, S. P. A.; Gentalen, E.; Ngo, N. *J. Am. Chem. Soc.* **1997**, *119*, 5081−5090.
(12) Zammatteo, N.; Jeanmart, L.; Hamels, S.; Courtois, S.; Louette, P.; Hevesi, L.; Remacle, J. *Anal. Biochem.* **2000**, *280*, 143−150.
(13) Diehl, F.; Beckmann, B.; Kellner, N.; Hauser, N. C.; Diehl, S.; Hoheisel, J. D. *Nucleic Acids Res.* **2002**, *30*, e79.
(14) Fahy, E.; Davis, G. R.; Dimichele, L. J.; Ghosh, S. S. *Nucleic Acids Res.* **1993**, *21*, 1819−1826.
(15) Benters, R.; Niemeyer, C. M.; Drutschmann, D.; Blohm, D.; Wöhrle, D. *Nucleic Acids Res.* **2002**, *30*, e10.
(16) Beier, M.; Hoheisel, J. D. *Nucleic Acids Res.* **1999**, *27*, 1970−1977.
(17) Le Berre, V.; Trévisiol, E.; Dagkessamanskaia, A.; Sokol, S.; Caminade, A.-M.; Majoral, J.-P.; Meunier, B.; François, J. *Nucleic Acids Res.* **2003**, *31*, e88.
(18) Benters, R.; Niemeyer, C. M.; Wöhrle, D. *ChemBioChem* **2001**, *2*, 686−694.

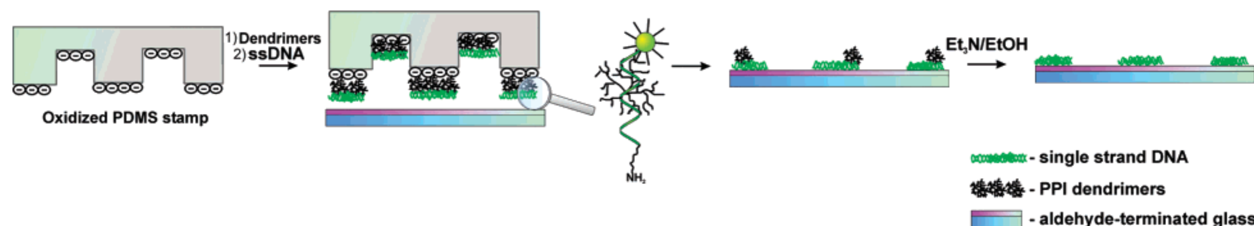

Figure 1. Schematic representation of microcontact printing of DNA with dendri-stamps. An oxidized PDMS stamp is first inked with dendrimers and subsequently incubated with fluorescein-labeled DNA. After transfer printing DNA onto the solid support, the substrate is rinsed with EtOH/Et_3N to wash residual dendrimers from the DNA substrate.

used as substrates for DNA microarrays. Typically, probes are modified at the 5′-terminus with a reactive group that can bind covalently with the support. Recently, methods such as dip-pen nanolithography[20] and nanografting[21] were introduced to extend the resolution of DNA arrays into the nanoscale.

Ideally, a DNA chip would have the following properties: (1) high and homogeneous probe density for optimal signal read-out, (2) submicron spot size and nanoscale spot resolution for high data density, (3) many thousands of different probes spotted identically and rapidly for large probe arrays; (4) simple, parallel manufacturing and analysis. It is evident that state-of-the-art DNA microarray technology falls short of this ideal.[6−9] Inhomogeneous spots result from printing from pins or pipettes due to the evaporation of solvent. Higher DNA concentrations remain at the edges ("doughnut effect") or DNA aggregates at a few points within a spot. Spot sizes are typically in the 50 micron range, spots are separated by at least 50 micron, and smaller spots can only be produced accurately by time-consuming serial processes. Soft lithography methods such as microcontact printing are not useful to pattern multiple probes simultaneously.[22−25]

In this paper, we report a simple but efficient method to transfer DNA as well as RNA to a glass substrate and facilitate positioning of oligonucleotides in molecular monolayers with submicron edge resolution by microcontact printing (μCP). An essential novelty of our method is the modification of PDMS stamps with fifth generation poly(propylene imine) (G5-PPI) dendrimers ("dendri-stamps"). In addition, we have combined microcontact printing with contact printing robotic systems to deposit hundreds of oligonucleotide spots and print them repeatedly from one stamp with good spot uniformity.

The key to this work is the electrostatic interaction between a negatively charged, oxidized PDMS stamp and positively charged G5-PPI. Dendrimers such as PPI and poly(amidoamide) (PAMAM) have become an interesting alternative for surface modifications and immobilization of biomolecules. Dendrimer-modified substrates were previously used for the attachment of DNA.[15−18] Dendron-modified glass slides were used to obtain controlled spacing between immobilized DNA molecules.[26,27]

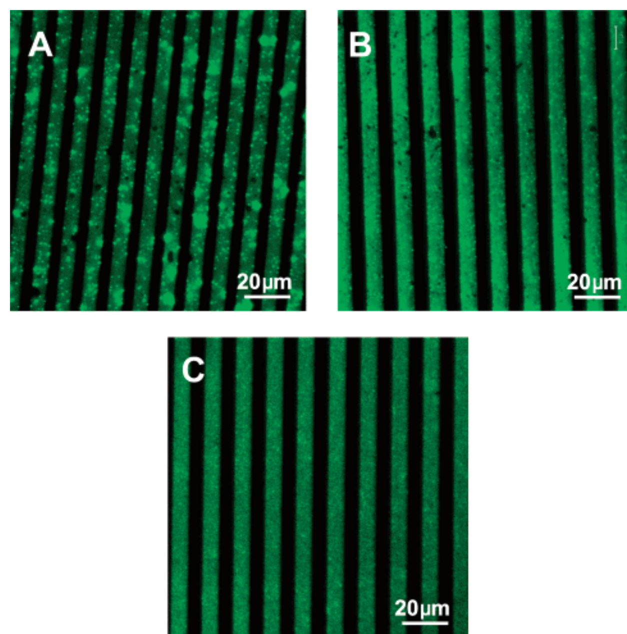

Figure 2. Patterns of fluorescein-labeled DNA patterns obtained by printing with dendri-stamps. An oxidized PDMS stamp was modified with (A) 5 mM, (B) 1 mM, and (C) 1 μM solution of PPI dendrimers.

"Dendri-slides" (dendrimers covalently attached to a glass substrate) generate a high surface coverage of oligonucleotides that can be further hybridized with high yield.[17] Grafting of DNA on such substrates resulted in uniform and high-density layers. Moreover, dendrimers were used as DNA transport agents in gene therapy to deliver genetic material to diseased sites.[28−30] G5-PPI possesses 64 amino groups at the periphery, providing a high density of terminal amino groups at the outer sphere.[31] At neutral pH, a significant fraction of the peripheral and internal amines are protonated, and the dendrimers can bind electrostatically to the negatively charged phosphate backbone of DNA.[28−33] All types of nucleic acids, including plasmid DNA, single-stranded and double-stranded oligonucleotides, and RNA, can form complexes via electrostatic interactions with PPI or PAMAM dendrimers.[30] The modification of PDMS

(19) Afanassiev, V.; Hanemann, V.; Wölfl, S. *Nucleic Acids Res.* **2000**, *28*, e66.
(20) Demers, L. M.; Ginger, D. S.; Park, S. J.; Li, Z.; Chung, S. W.; Mirkin, C. A. *Science* **2002**, *296*, 1836−1838.
(21) Xu, S.; Liu, G. Y. *Langmuir* **1997**, *13*, 127−129.
(22) Lange, S. A.; Benes, V.; Kern, D. P.; Hober, J. K. H.; Bernard, A. *Anal. Chem.* **2004**, *76*, 1641−1647.
(23) Xu, C.; Taylor, P.; Ersoz, M.; Fletcher, P. D. J.; Paunov, V. *J. Mater. Chem.* **2003**, *13*, 3044−3048.
(24) Thibault, C.; Le Berre, V.; Casimirius, S.; Trévisiol, E.; François, J.; Vieu, C. *J. Nanobiotechnol.* **2005**, *3*, 7.
(25) Björk, P.; Holmström, S.; Inganäs, O. *Small* **2006**, *2*, 1068−1074.
(26) Hong, B. J.; Oh, S. J.; Youn, T. O.; Kwon, S. H.; Park, J. W. *Langmuir* **2005**, *21*, 4257−4261.
(27) Hong, B. J.; Sunkara, V.; Park, J. W. *Nucleic Acids Res.* **2005**, *33*, e106.
(28) Zinselmeyer, B. H.; Mackay, S. P.; Schatzlein, A. G.; Uchegbu, I. F. *Pharm. Res.* **2002**, *19*, 960−967.
(29) Braun, C. S.; Vetro, J. A.; Tomalia, D. A.; Koe, G. S.; Middaugh, C. R. *J. Pharm. Sci.* **2005**, *94*, 423−435.
(30) Heiser, W. *Methods in molecular biology. Gene delivery to mammalian cells. Vol. 1. Nonviral gene transfer technique*; Human Press: Totowa, NJ, 2004.
(31) Bosman, A. W.; Janssen, H. M.; Meijer, E. W. *Chem. Rev.* **1999**, *99*, 1995−1688.
(32) Kabanov, V. A.; Zezin, A. B.; Rogacheva, V. B.; Gulyaeva, Z. G.; Zansochova, M. F.; Joosten, J. G. H.; Brackman, J. *Macromolecules* **1999**, *32*, 1904−1909.
(33) Van Duijvenbode, R. C.; Borkovec, M.; Koper, G. J. M. *Polymer* **1998**, *39*, 2657−2664.
(34) Decher, G. *Science* **1997**, *277*, 1232−1237.

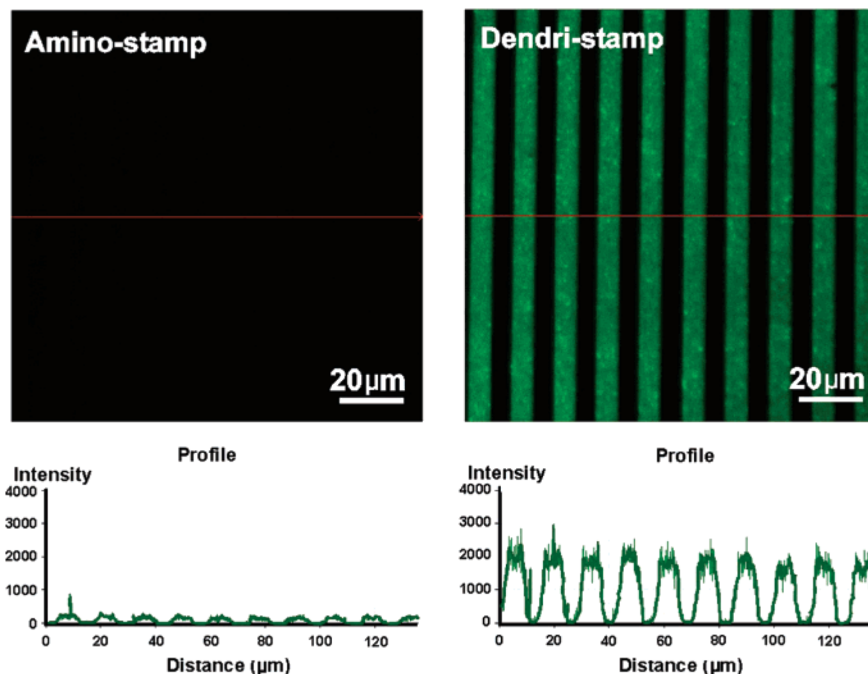

Figure 3. Patterns of fluorescein-labeled DNA patterns obtained by microcontact printing using APTES-modified PDMS stamp (left) and transfer printing with a dendri-stamp (right).

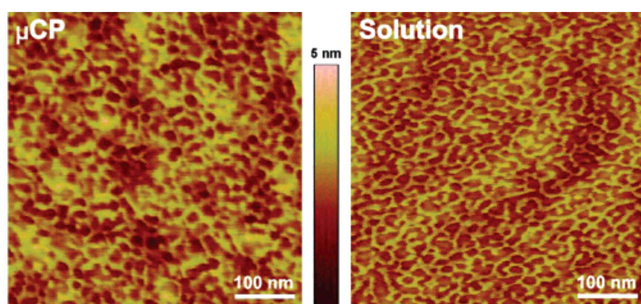

Figure 4. AFM tapping mode images of immobilized DNA using transfer printing with a dendri-stamp (left) and from solution (right).

stamps with G5-PPI ensures a high density of positive charge on the stamp surface that can attract negatively charged DNA and RNA molecules in a "layer-by-layer" arrangement.[34] PDMS stamps modified in this manner are able to transfer DNA to suitable solid supports creating patterns characterized by homogeneous distribution, high coverage and efficient hybridization. Here, we apply imine chemistry to bind covalently amino-modified DNA and RNA molecules to an aldehyde-terminated substrate. The labile imine bond is reduced to a stable secondary amine bond forming a robust connection between the oligonucleotide strand and the solid support.

Results and Discussion

The protocol of printing DNA using dendrimer-modified PDMS stamps ("dendri-stamps") is outlined in Figure 1. An oxidized PDMS stamp was modified with dendrimers by immersion in 1 μM ethanolic solution of G5 PPI dendrimers for 30 s and dried with N_2. 3′-Fluorescein-labeled, 5′-amino-functionalized single-stranded DNA (21 bases) was incubated

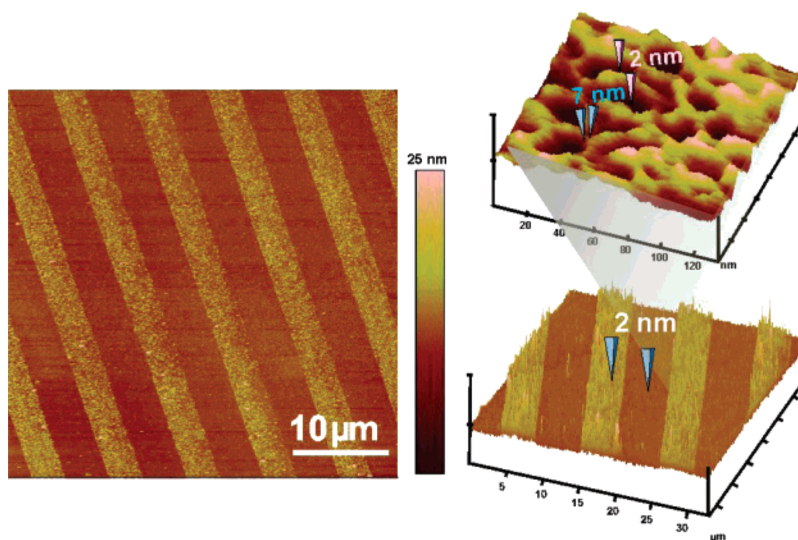

Figure 5. DNA pattern made by microcontact printing onto aldehyde-terminated glass support using dendri-stamps. The size of the DNA meshwork is 2 nm in height and 7 nm in width, and the height of the strand corresponds to the height of the pattern.

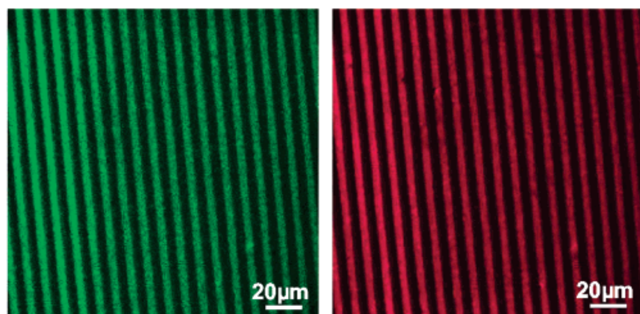

Figure 6. Simultaneous imaging of DNA patterns after hybridization between fluorescein-labeled probe (left) and Cy5-labeled target (right) by fluorescent confocal microscope.

on the modified PDMS stamp for 20 min at RT. The stamp was dried with N_2 and brought into conformal contact with the aldehyde-modified glass slide for a contact time of 15 s. The substrate was rinsed with ethanol containing a drop of Et_3N (to remove residual PPI dendrimer) and subsequently with water. To ensure that the imine linkage between the oligonucleotide strand and the substrate is not hydrolyzed, the imine bond was reduced to the corresponding amine by reaction with sodium borohydride. We have not observed loss of signal intensity of printed DNA due to the imine reduction reaction. To study the influence of the dendrimer concentration on the quality of microcontact printing, we prepared different concentrations of that solution: 5 mM, 1 mM, and 1 μM. Fluorescent images taken after transfer printing of DNA with the dendri-stamp and after imine reduction are shown in Figure 2.

Using the same settings and parameters for signal detection, stamps modified with the 1 μM solution of dendrimers showed the best results of the microcontact printed pattern. The pattern of printed oligonucleotides was homogeneous whereas the patterns obtained by printing with the stamps modified by the solutions with higher concentration of dendrimers show a heterogeneous density of oligonucleotides in the patterned area. This phenomenon could be related to the formation of aggregates between dendrimers and oligonucleotides. If there is an excess (i.e., more than a monolayer) of dendrimers on the stamp, they could possibly mix with deposited oligonucleotides and form aggregates ("polyplexes")[28-30] that are later difficult to remove just by rinsing.

DNA was also incubated on an oxidized PDMS stamp *without* modification with dendrimers. After printing *no* pattern was observed. Even though the oxidized stamp is hydrophilic it is also slightly negatively charged which might repel DNA. We also compared the transfer of DNA molecules with a dendri-stamp with a PDMS stamp that was modified with 3-aminopropyltriethoxysilane (APTES).[22] A solution of fluorescein-labeled oligonucleotides (1 μM in Tris-EDTA buffer) was incubated on top of the amino-modified PDMS stamps for 20 min at RT. Subsequently, the stamps were dried with nitrogen and brought into conformal contact with aldehyde-modified glass slides for 15 s. The substrates were washed with ethanol containing a drop of triethylamine, dried with nitrogen, and imaged with a laser-scanning confocal microscope. Both substrates were imaged using the same microscope settings (Figure 3). The intensity of the pattern made by printing oligonucleotides via the dendri-stamp is 10 times higher than that measured on the substrate printed via APTES-modified PDMS stamp. The higher probe density obtained with the dendri-stamp compared to the APTES-modified stamp is probably due to the higher surface concentration of positively charged amino groups that attract negatively charged oligonucleotides.

AFM was used to investigate the topography and distribution of DNA on the substrate after transfer printing with the dendri-stamp and after DNA immobilization from solution (i.e., the substrate was immersed in the solution of oligonucleotide for overnight, rinsed, and dried with nitrogen). The packing of DNA strands was almost identical (Figure 4), but the time necessary for the proper immobilization was drastically different - 15 s for μCP and 16 h for immobilization from solution. From AFM measurements it appears that during printing DNA strands are transferred and immobilized into a meshwork. These features are approximately 2 nm in height and 7 nm in width (Figure 5). From these AFM images, it can be concluded that only a

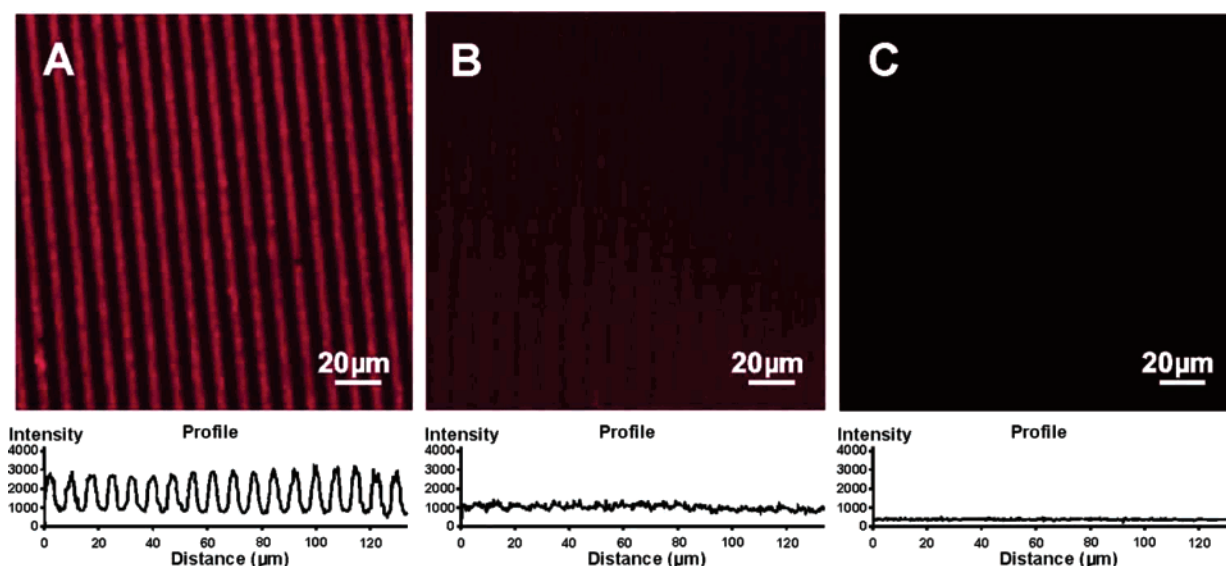

Figure 7. DNA patterns after hybridization of the transfer printed oligonucleotide with Cy5-labeled oligonucleotide having (A) complementary sequence, (B) sequence with one mismatch, and (C) noncomplementary sequence.

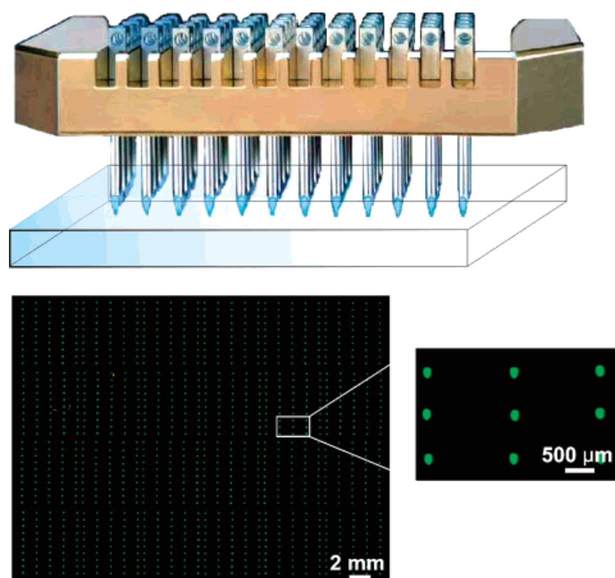

Figure 8. Robotic contact printing of DNA onto a flat dendri-stamp. Fragment of a fluorescence image of an aldehyde-terminated glass slide with an array of 400 spots of oligonucleotide labeled with fluorescein printed using a dendri-stamp.

monolayer of DNA is transferred from the dendri-stamp to the glass substrate.

To investigate whether the printed DNA is available for hybridization, the pattern was reacted with complementary Cy5-labeled oligonucleotides. It was also important to examine if dendrimers affect the conformation of DNA on the surface which subsequently might have an influence on hybridization. Hybridization with a complementary strand was carried out overnight in 1 μM solution in 4× standard saline citrate (SSC) containing 0.2% sodium dodecyl sulfate (SDS). Both strands—the probe (green fluorescein dye) and target (red Cy5 dye)—are distinctly visible in the fluorescence microscope (Figure 6).

To examine the specificity of the hybridization, we assayed the extent of mismatch by the reaction with a strand that differs just in one nucleotide in the middle of the sequence and with a strand that has a completely noncomplementary sequence. Printed oligonucleotides were exposed to Cy5-labeled oligonucleotides. The fluorescence signals acquired after hybridization show marginal signal with the mismatch oligonucleotide and no signal with the oligonucleotide probe of unrelated sequence (Figure 7). These experiments also confirm that no significant amount of dendrimer is left on the substrate because residual dendrimers would likely bind any nucleotide sequence in an unspecific (electrostatic) manner.

From the AFM and confocal fluorescence microscopy images, we conclude that transfer printing of DNA using a dendrimer-modified PDMS stamp results in faithful replication of the microscale topology of the stamp as a DNA micropattern on the substrate. The edge resolution of the DNA pattern is better than 1 micron. The DNA molecules form a dense monolayer on the substrate and are distributed homogeneously across the contact areas. The DNA probes are available for hybridization with complementary oligonucleotide strands.

To expand this method to multiple-probe printing, we have combined spotting (contact printing) of a stamp with microcontact printing. By using a robotic spotting system (MicroGrid, Apogent Discoveries), we have fabricated an array of amino-modified, fluorescein labeled oligonucleotide spots onto a flat dendri-stamp. The average spot size in this array is ca. 100 micron. The DNA-spotted dendri-stamp was brought into conformal contact with an aldehyde-terminated glass slide for 15 s contact time. The microarray on the stamp was replicated faithfully as a microarray on the aldehyde substrate. The spot size is regular, and the probe density is homogeneous (Figure 8).

In a second experiment, we have fabricated an array of an amino-modified oligonucleotide labeled with fluorescein and two mixtures of polyadenylated RNA labeled with Cy3 and Cy5, respectively, onto a flat dendri-stamp. The spotted dendri-stamp was brought into conformal contact with an aldehyde-terminated glass slide for 15 s contact time and subsequently used for 3 additional prints without supplementary reloading with oligonucleotides. After each microcontact print, the substrates were rinsed thoroughly with ethanol containing a drop of triethylamine, ethanol, and water. The first three prints exhibit almost equal intensities and densities of oligonucleotides and RNA mixtures on the aldehyde substrate. A significant decrease of intensity is observed for the fourth print (Figure 9). Microcontact printed spots of oligonucleotides and RNA mixtures on the aldehyde-terminated glass slides have a good spot uniformity. This experiment demonstrates that a microarray of hundreds of probes can be replicated faithfully several times by microcontact printing using the dendri-stamps and that DNA and RNA are both printed equally well.

Conclusion

We have developed a method to transfer DNA to a target substrate and to replicate a DNA microarray. We showed that by printing oligonucleotides using a dendri-stamp we can obtain a homogeneous pattern of the probe with a high-intensity signal. There is no difference between the edge and the middle of the pattern (the so-called “doughnut effect”). Oligonucleotides printed in 15 s are adsorbed with the same density as a probe that was immobilized from the solution during at least 16 h. In addition, patterns of oligonucleotides printed with a dendri-stamp show 10 times higher intensity than patterns obtained by printing DNA with an APTES-modified PDMS stamp. This suggests that also the probe surface concentration is much higher. DNA that is printed in this manner is available for hybridization. The fabrication of uniform patterns does not require specialized equipment, cleanroom facilities, complex technology, and time-consuming modifications of PDMS. This fabrication method of uniform DNA patterns can subsequently be combined with spotting systems which enable the deposition of hundreds of oligonucleotide spots on the same dendri-stamp. This array can be microcontact printed multiple times. From one “master” DNA array on the dendri-stamp it is possible to obtain 3−4 copies of that array with high DNA density and good spot homogeneity. Moreover, RNA molecules appear to be transferred equally well compared to DNA oligonucleotides. This method can be readily extended to arrays of many thousands of probes and should facilitate and improve microarray-fabricated substrates. We believe that this method can be also used in protein and carbohydrate microassay technology.

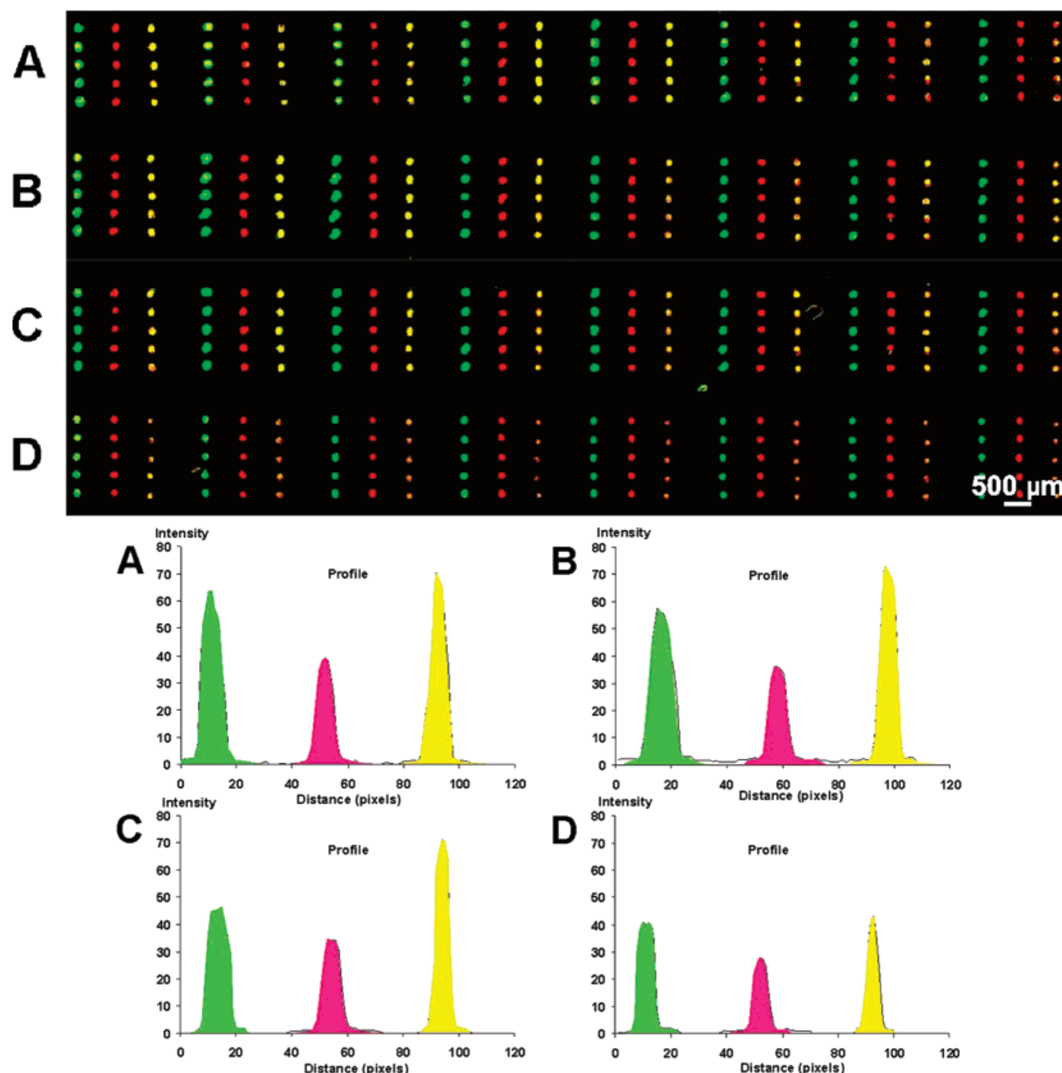

Figure 9. (Top) Fluorescent microscopy image of an array of four microcontact printed patterns (from A to B) that were fabricated using one dendri-stamp with spotted different oligonucleotides. Total number of spots: 120. (A) First print, (B) second print, (C) third print, and (D) fourth print. (Bottom) Average intensity profiles of three different dyes: fluorescein (green), Cy5 (red), Cy3 (yellow) attached to DNA (green), or RNA (red and yellow).

Experimental Section

Materials. All oligonucleotides were purchased from Sigma. The probe sequence employed in surface studies had sequence 5′-GTG CAC CTG ACT CCT GTG GAG-3′ (single strand of mutant β-globin gene) and was modified at the 5′ terminus with a six-carbon linker and amino group ($NH_2-(CH_2)_6-$) and at the 3′ terminus with fluorescein. The target sequence was 5′- CTC CAC AGG AGT CAG GTG CAC-3′, 5′-CTC CTC AGG AGT CAG GTG CAC-3′ (single mismatch), 5′-CAC GTG GAC TGA GGA ACA CCT-3′ (total mismatch, reverse sequence) all with the Cy5 modification at the 5′ terminus. All nucleotides were purified by HPLC and modified by the manufacturer. The probe concentration was 1 μM in Tris-EDTA buffer pH 8 and the target concentration was 1 μM in 4× SSC, 0.2% SDS solution. Before use, oligonucleotides were denatured at 95 °C for 5 min. All buffers and immobilization solutions were prepared with 18 MΩ·cm distilled water (MilliQ). The following materials and chemicals were used as received: poly(dimethylsiloxane) (PDMS) (Dow Corning), $NaBH_4$ (Aldrich), trimethoxysilylalkylaldehyde (Fluorochem), polypropyleneimine tetrahexacontaamine dendrimers, generation 5 (Aldrich), 3-aminopropyltriethoxysilanes (APTES) (Aldrich). All solvents were HPLC grade, and all other reagents were analytical grade. Other solvents or reagents were purchased from either Aldrich or Sigma.

Modification of Glass Slides. Clean microscope cover glass (Paul Marienfeld Gmbh & Co. KG, Germany) was activated with piranha solution for 45 min (concentrated H_2SO_4 and 33% aqueous H_2O_2 in a 3:1 ratio) (*Warning! Piranha solution should be handled with caution: it has been reported to detonate unexpectedly!*), rinsed with water (MilliQ), and immediately immersed in 0.1 vol % trimethoxysilylalkylaldehyde in toluene for 1 h. Following monolayer formation, the substrates were rinsed with toluene to remove any excess of silanes and subsequently dried in N_2.

Modification of Gold Substrates. Aldehyde-terminated monolayers on annealed gold substrates were prepared according to previously published procedures.[35]

Fabrication of Stamps. Silicon wafer-based masters with etched structures were prepared by UV photolithography. The master surface was fluorinated using fluorosilanes. PDMS stamps were fabricated by curing Sylgard 184 on the surface of the master at 60 °C for 12 h.

Microcontact Printing of DNA with Dendrimers. PDMS stamps were first oxidized in UV/plasma reactor (Ultra-Violet Products, model PR-100) for 30 min at a distance of about 2 cm from the plasma source. This reactor contains a low-pressure mercury UV light operating with UV emissions at 185 nm (1.5 mW cm^{-2}) and 254 nm (15 mW cm^{-2}).

(35) Rozkiewicz, D. I.; Ravoo, B. J.; Reinhoudt, D. N. *Langmuir* **2005**, *21*, 6337−6343.

Subsequently the hydrophilic stamps were immersed in 1 μM ethanolic solution of dendrimers for 30 s and blow dried with nitrogen. A drop of oligonucleotide solution was incubated on the stamp for 20 min at room temperature. The probe concentration was 1 μM in Tris-EDTA buffer pH 8 solution. The stamp was dried with nitrogen and brought into conformal contact with the aldehyde-terminated glass slide for 15 s. After printing, the stamp was lifted off and the substrate was rinsed with 30 mL of ethanol containing a drop of triethylamine to remove the dendrimer layer and subsequently dried with nitrogen.

Reduction of Imines on the Surface. After printing amino-modified oligonucleotides onto the aldehyde-terminated substrate, the glass slide was immersed in solution containing 50 mg $NaBH_4$ in 30 mL PBS with 10 mL EtOH for 5 min as described by Afanassiev et al.[19] After reaction time the substrate was washed in 0.2% SDS solution for 2 min under agitation, in water for 1 min and subsequently dried with nitrogen.

Hybridization on the Substrate Surface. For hybridization, a 5′-Cy5-labeled oligonucleotide was diluted to 1 μM in 4× SSC containing 0.2% SDS and applied to the surface of the modified glass slide as described by Afanassiev et al.[19] A coverslip was mounted gently on the top of the solution and the substrates where transferred to the hybridization oven at 42.3−47.3 °C for overnight. The excess, nonhybridized probes were removed by washing with vigorous agitation in the 1× SSC with 0.1% SDS solution for 5 min at hybridization temperature, 0.1× SSC with 0.1% SDS for 5 min at RT, and subsequent washing in water for 5 min. After washing, the glass slides were dried with nitrogen and scanned on a confocal fluorescent microscope (Zeiss 510) to visualize hybridization signals.

Atomic Force Microscopy (AFM). AFM measurements were carried out on a Dimension 3100/Nanoscope IVa (Digital Instruments, Santa Barbara, CA) in tapping mode, with 512 × 512 data acquisitions, using ultrasharp tips (MikroMash, Spain). All imaging was conducted at room temperature in air. Annealed gold substrates with aldehyde-terminated thiol monolayers[35] were used as substrates for AFM imaging.

Fluorescence Microscopy. Fluorescent images were acquired with a Carl Zeiss LSM 510 scanning confocal microscope. Red- and green-labeled DNA were visualized with λ_{ex} = 650 nm (λ_{em} = 670−700 nm) and λ_{ex}= 495 nm (λ_{em} = 517 nm), respectively. The emitted fluorescence was collected on a R6357 spectrophotometer.

Contact Printing by Robotic Systems. The spots of oligonucleotides were fabricated using MicroGrid, Apogent Discoveries. Flat, 2−3 mm thin dendri-stamps were used as substrates for contact printing. The following probes were used for spotting experiment: 5′-NH_2−C_6-GTG CAC CTG ACT CCT GTG GAG-fluorescein-3′, and mixtures polyA-RNA of *Bacillus subtilis* genes (ycxA, yceG, ybdO, ybbR, ybaS, ybAF) labeled with Cy-5 and (ybaC, yacK, yabQ, Trp, Thr, Dap, Phe, Lys) labeled with Cy-3 using the ULS labeling system (Kreatech BV, Amsterdam). The spotting solution of probe was 1 μM in Tris-EDTA buffer pH 8 solution. After printing, the substrates were left to dry under ambient conditions and used for microcontact printing.

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