DOI: 10.1002/adma.200501170

Dendrimer-Scaffold-Based Electron-Beam Patterning of Biomolecules**

By *Parijat Bhatnagar*, *Sonny S. Mark*, *Il Kim*, *Hongyu Chen*, *Brad Schmidt*, *Michal Lipson*, and *Carl A. Batt**

Microarrays have revolutionized the field of genomics and more recently proteomics and have proven to be an asset in high-throughput screening.[1–3] As the demand for improved sensitivity and throughput of biomolecular assays increases, considerable research effort has been put into developing microelectronic[4–9] and nanophotonic[10–12] biosensors, which are presumably more sensitive than conventional fluorescence-based assays and have a faster response time. The lithographic technology for making the densely packed microelectronic devices in a high-throughput manner is already quite advanced and may be integrated to form biosensors. This is expected to increase the pace of research in early detection of disease biomarkers,[5–7,12–17] discovering cell-signal transduction pathways,[15–22] and in drug discovery.[1,23,24] Denser arrays are also important for reducing reagent volume consumption and to improve sensitivity.[25] Successful biomolecule patterning on sensor chip circuitry requires a number of important steps. First, selective immobilization of the probe and reduction in non-specific binding should be achieved for higher signal-to-noise ratios. Second, reduction in the sensor size reduces the background, enhancing the signal-to-noise ratio, and therefore biomolecule patterns on the same order as the sensors are desirable. Third, the biomolecule pattern should be aligned with the sensor circuitry, which becomes more difficult as sensor size decreases. Finally, a fabrication process should be formulated to ensure that the biomolecules are intact and functional, which is a challenge given the harsh micro- or nanofabrication processing steps.[26] Here, we have demonstrated an electron-beam (e-beam)-based approach fulfilling the above requirements for patterning biological macromolecules that does not involve the use of resist, hence eliminating the exposure of these biomolecules to harsh resist-stripping processes that are normally employed to remove the resist. A non-biofouling poly(ethylene glycol) self-assembled monolayer (PEG-SAM) was selectively removed by e-beam and patterned with aldehyde-terminated polyamidoamine dendrimer (ald-PAMAM-SAM) in a layer-by-layer (LbL) manner to covalently immobilize the aminated oligonucleotide, which bind only to their complementary sequence targets and can be stripped and reprobed. The Generation-6 (G-6) PAMAM molecule, terminated with 256 primary amine groups and 6.7 nm in diameter,[27] was used to increase the surface density of aldehyde functional groups to increase the oligonucleotide-immobilization efficiency.[28–31]

Current techniques for patterning biomolecules involve the use of polymer-based templates,[32–35] which can be removed mechanically without the use of organic solvents after biomolecule immobilization. However, serious limitations exist in each case. Poly(dimethylsiloxane) (PDMS)-based soft-lithographic techniques[32–34] cannot be used to create high-resolution patterns,[36] as aligning the PDMS pattern with sub-micrometer features has been shown to work in a mix-and-match[37] manner with an accuracy of only 2 μm. Although alignment is not an issue for biomolecule patterning based on polymer lift-off,[35] as it is an integrated process, this method involves extra steps of deposition and etching a polymer film, which increases the complexity of the process. Challenges are also encountered as the size of the photolithographic patterns decrease due to the increase in line-edge roughness (LER)[38,39] and the isotropic nature of oxygen plasma etch.[39] Patterned gold has been used for creating protein patterns using thiol-based linkers,[40] but gold surfaces cannot be tolerated in some biosensors[4–6,10] as gold interferes with the optical signal or conductivity of the sensor. This technique also includes extra photolithographic and lift-off processing steps for patterning gold. Protein patterning using fluorescence-tagged proteins

[*] Prof. C. A. Batt, P. Bhatnagar
Department of Biomedical Engineering, Cornell University
Ithaca, NY 14853 (USA)
E-mail: cab10@cornell.edu
Prof. C. A. Batt, S. S. Mark
Department of Microbiology, Cornell University
Ithaca, NY 14853 (USA)
Prof. C. A. Batt, Prof. I. Kim, H. Chen
Department of Food Science, Cornell University
Ithaca, NY 14853 (USA)
B. Schmidt, Prof. M. Lipson
Department of Electrical and Computer Engineering
Cornell University
Ithaca, NY 14853 (USA)

[**] We thank Leonardo Damasceno, Michael Guillorn, Xin Yang, Wageesha Senaratne, and Yajaira Sierra for technical discussions. I. K. also thanks L. G. Culture for sabbatical support. The authors would like to thank the support of National Science Foundation (NSF) (Grant ECS-0330110). This work was performed in part at the Cornell NanoScale Science and Technology Facility (CNF), a member of the National Nanotechnology Infrastructure Network, which is supported by the NSF (Grant ECS 03-35765). Additional work was performed at the Nanobiotechnology Center (NBTC) at Cornell University, an STC program of the NSF under Agreement No. ECS-9876771. Supporting Information is available online from Wiley InterScience or from the author.

ADVANCED MATERIALS

physically adhered to nanoparticles and assembled inside 60 μm etched features on a wafer[41] have been demonstrated; however, patterning in smaller dimensions with this technique is also limited by the LER obtained in the photolithographic dimensions of the etched features and, to some extent, nanoparticle size. E-beam patterning of octadecyltrimethoxysilane (ODS)-SAM was performed to create patterns of DNA;[42,43] however, ODS-SAM is not a preferred surface due to the non-specific adsorption of biomolecules on hydrophobic surfaces.[44] Dip-pen nanolithography[45–49] and nanografting[50,51] approaches provide impressive resolution but the technology is not mature enough for these to be used at the industrial scale, although some effort has been put in this direction.[52]

Figure 1 illustrates the LbL assembly technique we employed for preparing ald-PAMAM-SAM. First, a PEG-SAM layer was assembled in the vapor phase on a silicon wafer with 20 nm of thermal silicon oxide; this layer was then patterned

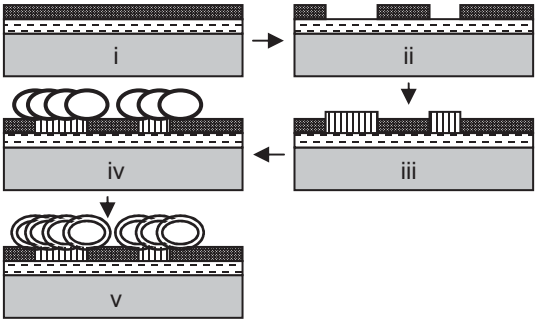

Figure 1. LbL method to pattern ald-PAMAM-SAM surrounded by PEG-SAM: i) Vapor-phase assembly of PEG-SAM, ii) e-beam patterning, iii) liquid-phase assembly of ald-SAM, iv) immobilization of G-6 PAMAM-SAM, v) modification with glutaraldehyde to create ald-PAMAM-SAM.

by an e-beam aligned to pre-existing features on the silicon oxide to regenerate the silanol groups. In general, the e-beam system can be used to achieve pattern alignment within 30 nm across the sample. Next, an aldehyde terminated SAM (ald-SAM) was assembled on the above regenerated silanol groups in the liquid phase. This was followed by the assembly of G-6 PAMAM on ald-SAM using Schiff base reaction between the aldehyde groups on ald-SAM and the primary amine groups on the PAMAM to create PAMAM-SAM. Schiff base reaction was again utilized to assemble glutaraldehyde on PAMAM-SAM to create aldehyde terminated PAMAM-SAM in the e-beam defined patterns (ald-PAMAM-SAM). Dendrimer-activated surfaces have been demonstrated for higher immobilization efficiencies and lower detection limits.[28–31] Here, PAMAM-SAM served to increase the surface density of the reactive aldehyde groups and also acted as a 6.7 nm spacer[27] for easier access of target DNA towards immobilized probe DNA. Figures S1,S2 (Supporting Information) show the preparation scheme of two control surfaces containing ald-SAM patterns surrounded by either a (1*H*,1*H*,2*H*,2*H*-perfluorooctyl) trichlorosilane (FOTS)-SAM background or a hexamethyldisilazane (HMDS)-SAM background. For both samples, vapor-phase assembly of the FOTS and HMDS was employed. These control samples were made to compare the non-specific adhesion of DNA on the FOTS-SAM or HMDS surface when compared with the PEG-SAM surface.

After exploring several aqueous and non-aqueous buffer conditions for optimizing the immobilization of the PAMAM dendrimers on the ald-SAM, we found that PAMAM dissolved in methyl alcohol with 0.08 % (v/v) acetic acid gave the most satisfactory results. This was done by checking the fluorescence emanating from probe DNA immobilized on the above-prepared ald-PAMAM-SAM.

Figure 2a shows a fluorescence image of the 1 μm patterns obtained after immobilizing carboxytetramethylrhodamine (TAMRA)-labeled probe DNA (pDNA) on the ald-PAMAM-SAM using Schiff base chemistry under aqueous conditions (pH 7.3) followed by reductive amination. Due to the resolution of fluorescence microscopy, we were not able to

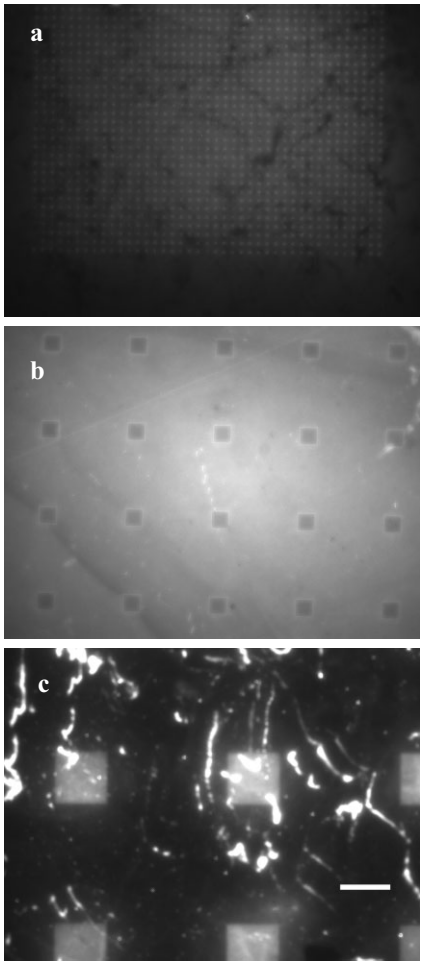

Figure 2. Aminated probe DNA immobilized on patterns of a) ald-PAMAM-SAM surrounded by PEG-SAM assembled in the vapor phase, b) ald-SAM surrounded by FOTS-SAM assembled in the vapor phase, and c) ald-SAM surrounded by PEG-SAM assembled in the liquid phase. Scale bar represents 30 μm in all three panels.

optically image the 30 and 300 nm patterns. The fluorescence image shows the expected uniform pattern of squares with minimal background noise. However, when substrates with pDNA patterns against a background of highly hydrophobic FOTS-SAM (or HMDS-SAM) (prepared as per Figs. S1,S2) were used, severe non-specific adhesion of pDNA was observed, as shown in Figure 2b. The high background noise observed for both FOTS and HMDS-SAM substrates is not completely unexpected, as hydrophobic surfaces are generally known to be susceptible to non-specific binding of biological molecules.[44] In an attempt to reduce the level of non-specific adhesion on the FOTS and HMDS surfaces, 0.5 % Triton X-100 (non-ionic surfactant)[44] (Sigma–Aldrich Corp., St. Louis, MO) was added to the pDNA solution during the probe immobilization step. However, the addition of Triton X-100 only resulted in the total elimination of any visible fluorescence patterns (not shown), indicating that the pDNA failed to immobilize on the patterned ald-SAM and ald-PAMAM-SAM areas. This result suggests that the aldehyde surfaces have become completely covered with surfactant, thus preventing covalent linkage with the pDNA. Subsequent washing steps with 0.5 % Triton X-100 in an ultrasonic bath to remove non-specifically adhered pDNA were also not successful. This is in confirmation with previous studies.[44] A significant amount of non-specific fluorescence was also observed when liquid-phase silanization was used to create PEG-SAM using long-chain (6–9 PEG units) 2-[methoxy(polyethyleneoxy)propyl] trimethoxysilane (MPEGTMS) (Gelest, Inc., Morrisville, PA) (Fig. 2c). This may be due to poor coverage of the surface by the PEG-SAM when using a liquid-phase versus a vapor-phase silanization protocol.[53,54] A larger pattern is shown in Figures 2b,c, as small patterns were not clearly discernible due to poor contrast from the high non-specific fluorescence background. Further control experiments were performed whereby the aldehyde functional groups on the ald-PAMAM-SAM were first quenched with a 0.05 M Tris/0.4 M glycine/0.05 M $NaCNBH_3$ buffer (Tris: tris(hydroxymethyl)aminomethane) before exposure to the pDNA solution. No fluorescence was observed in this case (not shown), demonstrating that the pDNA does not adhere non-specifically to the aldehyde surface.

Hybridization and selectivity assays were performed to test the functionality of the immobilized pDNA molecules by incubating the chips with either complementary target DNA (ctDNA) or non-complementary target DNA (ntDNA) tagged with Cy5 dye. Figure 3a shows a representative fluorescence image of ctDNA after hybridization to the pDNA immobilized on the dendrimer-activated patterns. No fluorescence was observed from pDNA pattern containing chips hybridized with ntDNA (negative control, not shown), proving that the immobilized pDNA was functional and retained its specificity towards its complementary target. In other negative control tests (not shown), a chip without any immobilized pDNA was hybridized with ctDNA; also, another chip with immobilized pDNA was exposed to a buffer solution without any ctDNA present. No fluorescence was observed in either case. These results show that ctDNA is not binding non-specifically to the ald-PAMAM-SAM, and that the pDNA does not give a false positive Cy5 signal due to the presence of the TAMRA fluorophore label.

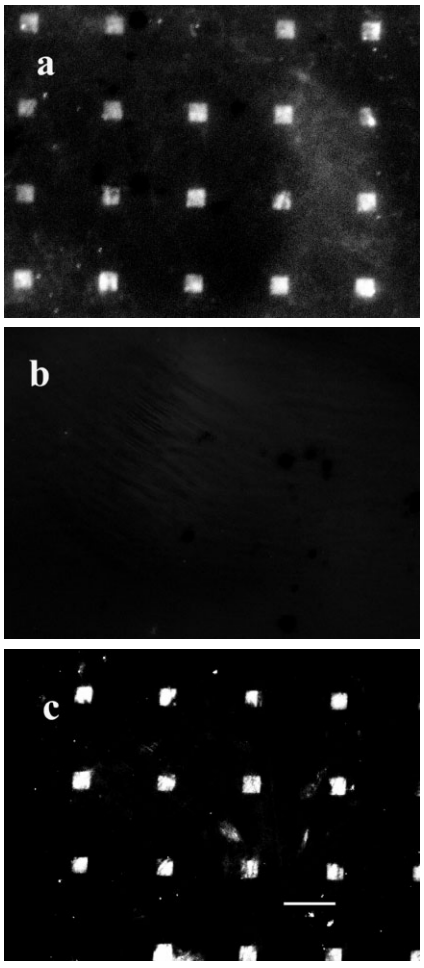

Figure 3. Hybridization assay of probe DNA with complementary target DNA. a) After hybridization with ctDNA. b) After treatment with 99.5 % formamide as a chaotropic medium. c) After rehybridization with ctDNA. Scale bar represents 30 μm for all three panels.

To test the reusability of the patterned surfaces with the pDNA, hybridized DNA complexes were first denatured by incubating in formamide solution for 10 min at room temperature and then rinsed in deionized water. This resulted in the removal of the bound ctDNA target and thus a complete loss of any visible Cy5 fluorescence (Fig. 3b). We found that exposure to the formamide for a period less than 5 min was insufficient for complete removal of the hybridized ctDNA. When the regenerated ("stripped") chip was again exposed to the ctDNA solution under the prescribed hybridization conditions, the Cy5 fluorescence patterns re-emerged (Fig. 3c) and appeared similar to those shown in Figure 3a. We also tried 8.3 M urea as the chaotropic medium to strip the hybridized ctDNA, but found that it required 30 min for complete stripping. The use of 8.3 M urea as the stripping reagent was also

ADVANCED MATERIALS

not advisable as it attacked the PEG-SAM, probably due to its basic nature, which resulted in specks of non-specific binding of ctDNA during the rehybridization step (not shown).

We have demonstrated here a biomolecular patterning technique capable of creating reusable DNA patterns using an e-beam process, without the use of a conventional e-beam resist, in 1 μm dimensions with respect to pre-existing micrometer-sized alignment features made by photolithography.[10] However, based on the accuracy of the e-beam tool, we suggest that pattern size and an alignment accuracy of 30 nm should be easily achievable. The process eliminates any requirement for a separate resist-removal step, and therefore exposure of the biomolecules to harsh chemical processing conditions during nanofabrication is avoided. This method uses the commercially available micro- and nanofabrication processes of vapor deposition[55] and e-beam lithography,[56,57] which can be easily integrated with fabrication steps for microelectronic or nanophotonic lab-on-a-chip type devices with biosensing capabilities.

Experimental

Preparation of PEG-SAM: A silicon wafer with 20 nm thermal SiO_2 was plasma cleaned, and PEG-SAM was assembled in the vapor phase at a chamber pressure of 0.5 torr using short-chain (single PEG unit) 2-[methoxy(polyethylenoxy)propyl] trichlorosilane (MPEGTCS) (MVD-100, Applied Microstructures, Inc., San Jose, CA) [55]. The process was repeated four times for 10 min each. The wafer was then rinsed in 2-propanol and cured overnight.

E-Beam Patterning of PEG-SAM: The PEG-SAM on the above wafer was then removed by e-beam lithography in patterns using a dose of 9 mC cm^{-2} using an accelerating voltage of 100 kV at a current of 20 nA with an approximate spot size of 20 nm (JBX-9300FS, JEOL USA, Inc., Peabody, MA) [56]. This was then rinsed in 2-propanol and blow dried in a N_2 jet.

Preparation of ald-SAM: The PEG-SAM on the wafer was removed by a 9 mC cm^{-2} e-beam and rinsed in 2-propanol. This was then treated ultrasonically for 2 h with a 3 % (v/v) solution of 11-triethoxysilylundecanalsilane (TESU) (Gelest, Inc., Morrisville, PA) dissolved in a stock solution of 95 % ethanol, 4.7 % water, and 0.3 % acetic acid and cured at 120 °C for 2 min.

Preparation of PAMAM-SAM: The wafer with ald-SAM was incubated for 2 h with a 0.05 % (w/v) solution of G-6 PAMAM dendrimer (Sigma–Aldrich Corp., St. Louis, MO, Product no. 536717) in methanol with 0.08 % (v/v) acetic acid.

Preparation of ald-PAMAM-SAM: The wafer with PAMAM-SAM was washed in methanol and incubated in a dilute solution of 7 % (w/v) glutaraldehyde (Sigma–Aldrich Corp., St. Louis, MO, Product no. G7776) in 97 % methanol and 3 % water for 2 h.

Probe DNA Immobilization: The above wafer was washed in methanol, and an aliquot of 5 μM pDNA (Integrated DNA Technologies, Coralville, IA) in phosphate-buffered saline (PBS) with 0.05 M $NaCNBH_3$ at pH 7.3 was incubated on ald-PAMAM-SAM for 5 min and rinsed with deionized water. The pDNA was functionalized with a free amino group (linked to a six-carbon chain spacer arm) at the 5′ end, tagged with a TAMRA fluorescent dye label on the 3′ end (5′-NH_2-C6-CAA GAT CGC ACT CCA GCC AG-TAMRA-3′), and bound to aldehyde groups with its 5′ end by Schiff base reaction followed by reductive amination. Any free reactive aldehyde groups remaining on the surface after probe immobilization were quenched with 0.05 M Tris/0.4 M glycine/0.05 M $NaCNBH_3$ buffer wash for 20 min.

Target DNA Hybridization: Silicon wafer pieces containing pDNA were incubated with a 2× SSPE (300 mM NaCl, 20 mM NaH_2PO_4, 2 mM EDTA, pH 7.4) buffer solution containing either 5 μM ctDNA (Integrated DNA Technologies, Coralville, IA) (5′-Cy5-TGT ACC GTA CCT GGC TGG AGT GCG ATC TTC-3′) or 5 μM ntDNA (5′-Cy5-GGG AAA AGG GAT CCG AAA AAA AGG GGT ACG-3′) for 30 min and subsequently washed with deionized water.

Denaturation of Hybridized DNA Complexes and Chip Regeneration: Chips containing hybridized ctDNA patterns were incubated in a 99.5 % solution of formamide (Sigma–Aldrich Corp., St. Louis, MO, Product no. F9037) for 10 min (25 °C) in order to denature double-stranded DNA complexes, and then rinsed with deionized water.

Fluorescence Imaging: An epifluorescence microscope (Labophot-2, Nikon, Inc., Melville, NY) fitted with a charge-coupled device (CCD) camera (Spot RT, Diagnostic Instruments, Inc., Sterling Heights, MI) was used for imaging of TAMRA- and Cy5-labeled oligonucleotides immobilized on the chip. All images were taken under the settings of a 20 s exposure time and a gain of two. No filter was used in the camera, and only the respective filter cube in the microscope for TAMRA (excitation: 541–551 nm, dichroic mirror: 575 nm, emission: 590 nm) or Cy5 (excitation: 590–650 nm, dichroic mirror: 660 nm, emission: 663–735 nm) was in place. For Cy5 imaging, a background image taken through a Cy5 filter on a separate chip without the ctDNA hybridization step was subtracted from the image of the chip containing hybridized Cy5-labeled ctDNA.

Received: June 7, 2005
Final version: September 22, 2005
Published online: January 10, 2006

[1] O. E. Beske, S. Goldbard, *Drug Discovery Today* **2002**, *7*, S131.
[2] J. Piehler, *Curr. Opin. Struct. Biol.* **2005**, *15*, 4.
[3] J. Owens, *Nat. Rev. Drug Discovery* **2004**, *3*, 911.
[4] K. Ramanathan, M. A. Bangar, M. Yun, W. Chen, N. V. Myung, A. Mulchandani, *J. Am. Chem. Soc.* **2005**, *127*, 496.
[5] Y. Cui, Q. Q. Wei, H. K. Park, C. M. Lieber, *Science* **2001**, *293*, 1289.
[6] W. U. Wang, C. Chen, K. H. Lin, Y. Fang, C. M. Lieber, *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 3208.
[7] D. G. Georganopoulou, L. Chang, J. M. Nam, C. S. Thaxton, E. J. Mufson, W. L. Klein, C. A. Mirkin, *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 2273.
[8] I. Tokareva, S. Minko, J. H. Fendler, E. Hutter, *J. Am. Chem. Soc.* **2004**, *126*, 15 950.
[9] E. Katz, I. Willner, *Angew. Chem. Int. Ed.* **2004**, *43*, 6042.
[10] B. Schmidt, V. Almeida, C. Manolatou, S. Preble, M. Lipson, *Appl. Phys. Lett.* **2004**, *85*, 4854.
[11] R. P. Van Duyne, *Science* **2004**, *306*, 985.
[12] A. J. Haes, L. Chang, W. L. Klein, R. P. Van Duyne, *J. Am. Chem. Soc.* **2005**, *127*, 2264.
[13] L. A. Liotta, V. Espina, A. I. Mehta, V. Calvert, K. Rosenblatt, D. Geho, P. J. Munson, L. Young, J. Wulfkuhle, E. F. Petricoin, *Cancer Cell* **2003**, *3*, 317.
[14] H. M. Cui, M. Cruz-Correa, F. M. Giardiello, D. F. Hutcheon, D. R. Kafonek, S. Brandenburg, Y. Q. Wu, X. B. He, N. R. Powe, A. P. Feinberg, *Science* **2003**, *299*, 1753.
[15] M. F. Clarke, *Nature* **2004**, *432*, 281.
[16] S. K. Singh, C. Hawkins, I. D. Clarke, J. A. Squire, J. Bayani, T. Hide, R. M. Henkelman, M. D. Cusimano, P. B. Dirks, *Nature* **2004**, *432*, 396.
[17] M. Ferrari, *Nat. Rev. Cancer* **2005**, *5*, 161.
[18] S. Takayama, E. Ostuni, P. LeDuc, K. Naruse, D. E. Ingber, G. M. Whitesides, *Nature* **2001**, *411*, 1016.
[19] C. S. Chen, M. Mrksich, S. Huang, G. M. Whitesides, D. E. Ingber, *Science* **1997**, *276*, 1425.
[20] S. Raghavan, C. S. Chen, *Adv. Mater.* **2004**, *16*, 1303.
[21] A. Folch, M. Toner, *Annu. Rev. Biomed. Eng.* **2000**, *2*, 227.
[22] B. Vogelstein, K. W. Kinzler, *Nat. Med.* **2004**, *10*, 789.

[23] W. H. Koch, *Nat. Rev. Drug Discovery* **2004**, *3*, 749.
[24] D. Sidransky, *Nat. Rev. Cancer* **2002**, *2*, 210.
[25] D. A. Markov, K. Swinney, D. J. Bornhop, *J. Am. Chem. Soc.* **2004**, *126*, 16659.
[26] N. L. Abbott, J. P. Folkers, G. M. Whitesides, *Science* **1992**, *257*, 1380.
[27] D. A. Tomalia, A. M. Naylor, W. A. Goddard, *Angew. Chem. Int. Ed. Engl.* **1990**, *29*, 138.
[28] R. Benters, C. M. Niemeyer, D. Wohrle, *ChemBioChem* **2001**, *2*, 686.
[29] S. Pathak, A. K. Singh, J. R. McElhanon, P. M. Dentinger, *Langmuir* **2004**, *20*, 6075.
[30] P. Angenendt, J. Glokler, J. Sobek, H. Lehrach, D. J. Cahill, *J. Chromatogr. A* **2003**, *1009*, 97.
[31] E. Trevisiol, V. Le Berre-Anton, J. Leclaire, G. Pratviel, A. M. Caminade, J. P. Majoral, J. M. Francois, B. Meunier, *New J. Chem.* **2003**, *27*, 1713.
[32] Y. N. Xia, G. M. Whitesides, *Annu. Rev. Mater. Sci.* **1998**, *28*, 153.
[33] G. M. Whitesides, E. Ostuni, S. Takayama, X. Y. Jiang, D. E. Ingber, *Annu. Rev. Biomed. Eng.* **2001**, *3*, 335.
[34] J. C. McDonald, G. M. Whitesides, *Acc. Chem. Res.* **2002**, *35*, 491.
[35] B. Ilic, H. G. Craighead, *Biomed. Microdevices* **2000**, *2*, 317.
[36] A. S. Blawas, W. M. Reichert, *Biomaterials* **1998**, *19*, 595.
[37] M. Tormen, T. Borzenko, B. Steffen, G. Schmidt, L. W. Molenkamp, *Appl. Phys. Lett.* **2002**, *81*, 2094.
[38] D. L. Goldfarb, A. P. Mahorowala, G. M. Gallatin, K. E. Petrillo, K. Temple, M. Angelopoulos, S. Rasgon, H. H. Sawin, S. D. Allen, M. C. Lawson, R. W. Kwong, *J. Vac. Sci. Technol. B* **2004**, *22*, 647.
[39] A. P. Mahorowala, K. Babich, Q. Lin, D. R. Medeiros, K. Petrillo, J. Simons, M. Angelopoulos, R. Sooriyakumaran, D. Hofer, G. W. Reynolds, J. W. Taylor, *J. Vac. Sci. Technol. A* **2000**, *18*, 1411.
[40] M. Veiseh, M. H. Zareie, M. Q. Zhang, *Langmuir* **2002**, *18*, 6671.
[41] C. Wang, Y. Zhang, *Adv. Mater.* **2005**, *17*, 150.
[42] T. Tanii, T. Hosaka, T. Miyake, G. J. Zhang, T. Zako, T. Funatsu, I. Ohdomari, *Appl. Surf. Sci.* **2004**, *234*, 102.
[43] G. J. Zhang, T. Tanii, T. Zako, T. Hosaka, T. Miyake, Y. Kanari, T. W. Funatsu, I. Ohdomari, *Small* **2005**, *1*, 833.
[44] R. J. Marsh, R. A. L. Jones, M. Sferrazza, *Colloids Surf. B* **2002**, *23*, 31.
[45] K. B. Lee, S. J. Park, C. A. Mirkin, J. C. Smith, M. Mrksich, *Science* **2002**, *295*, 1702.
[46] S. Kramer, R. R. Fuierer, C. B. Gorman, *Chem. Rev.* **2003**, *103*, 4367.
[47] D. J. Pena, M. P. Raphael, J. M. Byers, *Langmuir* **2003**, *19*, 9028.
[48] J. H. Lim, D. S. Ginger, K. B. Lee, J. Heo, J. M. Nam, C. A. Mirkin, *Angew. Chem. Int. Ed.* **2003**, *42*, 2309.
[49] L. M. Demers, D. S. Ginger, S. J. Park, Z. Li, S. W. Chung, C. A. Mirkin, *Science* **2002**, *296*, 1836.
[50] K. Wadu-Mesthrige, N. A. Amro, J. C. Garno, S. Xu, G. Y. Liu, *Biophys. J.* **2001**, *80*, 1891.
[51] K. Wadu-Mesthrige, S. Xu, N. A. Amro, G. Y. Liu, *Langmuir* **1999**, *15*, 8580.
[52] NanoInk Inc., 1335 Randolph St., Chicago, IL 60607, USA or http://www.nanoink.net (accessed September 2005).
[53] J. Duchet, B. Chabert, J. P. Chapel, J. F. Gerard, J. M. Chovelon, N. Jaffrezic-Renault, *Langmuir* **1997**, *13*, 2271.
[54] J. Duchet, J. F. Gerard, J. P. Chapel, B. Chabert, *Compos. Interfaces* **2001**, *8*, 177.
[55] Available from Applied Microstructures Inc., 4425 Fortran Drive, San Jose, CA 95134, USA (see http://appliedmst.com).
[56] Available from JEOL USA Inc., 11 Dearborn Road, Peabody, MA 01960, USA (see http://www.jeolusa.com/index.html).
[57] Available from Photronics Inc., 601 Millennium Drive, Allen, TX 75013, USA (see http://www.photronics.com).

