DNA on Surfaces

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Multiple Length-Scale Patterning of DNA by Stamp-Assisted Deposition**

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The adsorption of biopolymers on solid surfaces determines their functionality, thus affecting the response of biomedical devices based on hybrid biological/(in-)organic interfaces. In the case of DNA molecules, the control of their hierarchical organization at surfaces must be achieved. There is a gap in controlling the length scales in experiments with DNA molecules on surfaces. In experiments in which individual molecules are manipulated by local probes or optical tweezers[1] the molecules are randomly deposited, anchored, or passed in a flowing liquid in the proximity of a surface. Their precise position cannot be predefined, and the addressing of individual molecules relies on high-resolution microscopes. On the other hand, devices for diagnostics are based on specific oligonucleotide sequences patterned into arrays whose cells have a lateral size in the range of tens of micrometers. In order to hybridize with complementary target sequences, DNA single strands in the cells must retain their own degrees of freedom to as great an extent as possible. A major limitation to the quantitative application of bioarray diagnostics is the interference of steric hindrance in a solid monolayer with the kinetics of hybridization. Therefore devising techniques for patterning individual DNA molecules or oligonucleotide probes from solution onto planar surfaces is of great significance.[2] These techniques could also be used for patterning peptides, antibodies, and their aggregates for the fabrication of functional membranes, scaffolds for cell and tissue growth, biosensors with specific recognition, and rewritable memories.[3]

Several approaches have been used to control the organization of DNA molecules on a solid surface, mostly with the purpose to align individual molecules along the stretching direction. These include deposition of a DNA solution on polydimethylsiloxane (PDMS) stamp followed by transfer printing on mica,[4] molecular combing[5] by capillary flow, spin stretching,[6] and casting solutions on a surface prepatterned with PDMS.[7] Arrays of DNA have been patterned by dip-pen nanolithography,[8] deposition on prepatterned templates by electron-beam lithography,[9] drop projection,[10] and microcontact printing (μCP).[11] All of these

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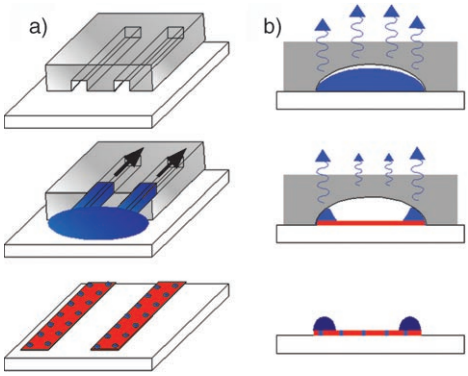
approaches yield micropatterned DNA surfaces. The only reports of ordered nanopatterns exploit deposition assisted by nanobeads[12] and templates made of anodic porous alumina.[13]

We report here on the multiple length-scale patterning of DNA on surfaces based on a printing technique coupled to dewetting. The results consist of nanodots on an area of a few mm^2, where each dot consists of a few DNA molecules. Our method exploits the self-organization of the DNA molecules in a solution confined between a stamp and the surface. The stamp imposes the larger length scale, and the self-organization brings about the smaller characteristic length scale[14] The shape, size, and spacing of DNA nanostructures can be modulated by the choice of stamp features and the concentration of the DNA solution, the latter controlling the wetting regime. For the first time dewetting is demonstrated as a viable route to the patterning of arrays of biomolecules.

Mica was used as a prototype surface for this experiment. Normally a solution of DNA in dilute buffer, such as that employed in the present study, does not wet mica, and when a drop of DNA buffer solution is cast on the mica surface, it generates a random aggregation of biomolecules and their counterions (Figure 1 a). This behavior is dictated by the electrostatic repulsion between the negatively charged DNA and the mica surface. The deposited DNA molecules are arranged randomly. This approach is not suitable for any application where localization of molecules at precise positions is required.

To overcome the problem of the placement of biomolecules, we applied micromolding in capillaries (MIMIC)[15] to confine the DNA solution within micrometric channels defined by the stamp protrusions in intimate contact with the surface. When the solution is placed at an open end of the cavity, the solution flows inside driven by capillary forces and surface tension with the boundary walls. Self-organization of the molecular solute occurs at the later stages of solvent evaporation. The process is depicted in Scheme 1, and an example of the resulting patterned DNA deposit is shown in Figure 1 b.

The large length scale is imposed by the periodicity of the stamp protrusions, while the smaller length scales, specifically the size and separation of the DNA deposits, are controlled by the wetting phenomena that occur in the space between the



Scheme 1. a) Deposition of DNA from solution by micromolding in capillaries (MIMIC) as described in the text. b) Pinning occurs during the last stages of solvent evaporation.

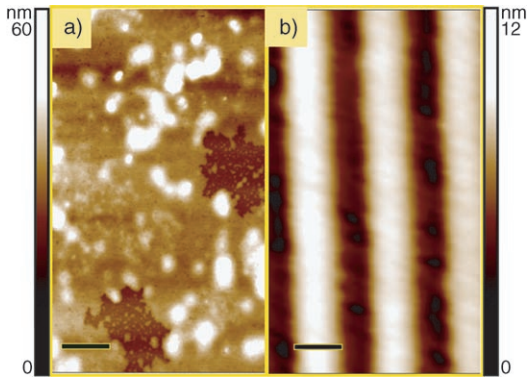


Figure 1. AFM images of 10 μL of a solution of λDNA on mica. Samples prepared by a) drop casting and b) patterning as shown in Scheme 1 a. Scale bar is 1 μm.

stamp and the surface. Depending on the concentration, the solute precipitates when the critical concentration is reached. If this occurs in when the channel is filled with a homogeneous layer of solution (Scheme 1 a), the solute precipitates in the form of a thin film. As the solution is pinned to the edges of the channel, the fluid section profile results in an inhomogeneous rate of evaporation of the solvent (Scheme 1 b). The convective flow of the solute towards the pinning sites results in the precipitation of split structures in the channel. The stamp was made of PDMS by replica molding.[15a] It consists of an array of parallel channels whose width and height are 1 μm and 230 ± 15 nm, respectively, and the pitch is 1.4 μm. The dimensional control can be achieved by changing the stamp features (periodicity and volume of the channels), the DNA concentration, and the volume of the infilling solution. Atomic force microscopy (AFM) was performed with an NT-MDT microscope in semicontact mode in air. λDNA solution (100–8000 bp, D9793 Sigma Aldrich) in 10 mM tris(hydroxymethyl)aminomethane hydrogen chloride (Tris-chloride, 93377 Sigma Aldrich) pH 7.4 buffer was used in our experiments. The stamp was placed onto freshly cleaved mica, and a 10-μL droplet of solution was deposited next to it and allowed to dry over approximately 12 h under ambient conditions. The stamp was then gently removed and the sample imaged.

When the concentration of the DNA solution was 1.25 $\mu g\,mL^{-1}$, the resulting pattern consisted of homogeneous lines (Figure 2 a) whose full width at half maximum is approximately 750 ± 50 nm, with a periodicity of 1.4 μm and an average height of 10 ± 1 nm. When a more concentrated solution was used, dots formed on top of a layer a few Å thick stretching along the length of the channels. For DNA concentrations between 2 and 4 $\mu g\,mL^{-1}$ the dots are roughly aligned but without well-defined spacing (Figure 2 b, c). When the concentration exceeded 5 $\mu g\,mL^{-1}$ spatial correlations emerge among the dots, and the dot size increases. In this case, the dots are perfectly aligned along the edges of the channel (Figure 2 d) as a result of the pinning of the solution and the convective flow from the center towards the edges. The height of the aligned hemispherically capped dots (full width at half maximum: 150 nm) is 18 ± 2 nm on average, at a distance of 600 ± 30 nm along and 1.4 μm perpendicular to the

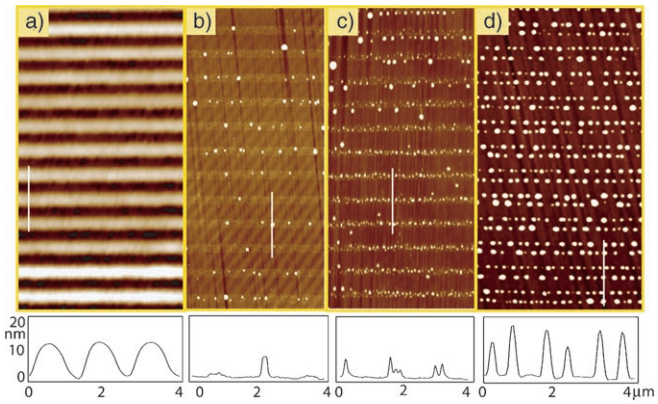


Figure 2. AFM images (above) and height profiles (below) of λDNA (100–8000 bp) patterned on a mica surface at different concentrations: a) 1.25 μg mL^{-1}, b) 2.0 μg mL^{-1}, c) 2.8 μg mL^{-1}, and d) 5.0 μg mL^{-1}.

patterned line. Whereas the height can be measured accurately, the full width at half maximum of the dots may be overestimated by nearly a factor of two because the tip convolution for curvature radii of tips is 10–20 nm.

To verify the results, we performed a control experiment by patterning 10 mM TRIS buffer alone; in this case stripes approximately 2 Å in height were observed. Moreover, we observed an ordered dot array also when the DNA concentration was kept constant at 5 μg mL^{-1} and the buffer concentration was varied between 20 and 100 mM. DNA is known to adopt coiled conformations at high concentration of salts, provided that they are salting-out or "structuring" salts.[16] The Tris-chloride system behaves as a neutral or slightly stabilizing salt. We therefore conclude that the arrays consist of DNA dots. We estimate that a dot of average volume, 3×10^5 nm^3, may contain up to 10000 base pairs, based on a density of 1.3 g cm^{-3} and considering DNA as the most abundant species in the dot.

Patterning occurs during the deposition of DNA molecules inside the channels. The DNA molecules, with a negatively charged shell, have a low affinity to the negatively charged mica surface, and the surface tension is a major driving force for the dot formation. In dilute DNA solutions the repulsive electrostatic interaction between the DNA molecules and the surface are shielded by the buffer, and a homogeneous continuous layer with salt and DNA is deposited. The transition from continuous lines to split strings of dots at higher DNA concentration arises from either dewetting of the initially continuous stripes,[17] or nucleation and growth in a partial wetting regime.[18] Both phenomena have been observed in thin films of molecular materials and are known to result in spontaneous spatial correlations in size and distance.[19] Dewetting is the rupture of an initially continuous film upon an external stimulation. It can develop by nucleation and growth of holes[20] or a spinodal mechanism,[21] which occurs by fluctuations of the film surface with the emergence of a characteristic wavelength. In the case of growth in partial wetting regime, correlations arise because of intertwined nucleation and ripening, the latter of which is a size- and distance-dependent phenomenon that stops when the nuclei have reached the same size at an equilibrium distance. In both regimes, the smaller length scales, dot size and spacing, depend on the initial concentration of the solution.

At high concentration, the dots are attracted to the corner formed by the stamp and the surface as a result of both capillary forces and the need to minimize their surface energy. The effect of surface tension and viscosity[22] on the channel-filling rate at increasing DNA concentration, as well as the interplay between the flow profile and deposition, are currently under investigation.

In summary, the possibility to pattern ordered arrays of nanostructures made of DNA has been demonstrated using a featureless stamp with micrometer channels. Our results hint at the possibility of the patterning of DNA for many applications by stamp-assisted deposition from a solution onto a substrate in a regime of partial wetting or dewetting. Our method is suitable for upscaling the deposition onto large areas. The control of multiple length scales by exploiting confinement and competing interactions between the adsorbate and the substrate represents a remarkable example of integrated top-down/bottom-up processes.

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[1] C. Bustamante, Z. Bryant, S. B. Smith, *Nature* **2003**, *424*, 338–341.
[2] A. A. Yu, T. Savas, S. Cabrini, E. diFabrizio, H. I. Smith, F. Stellacci, *J. Am. Chem. Soc.* **2005**, *127*, 16774–16775.
[3] J. S. Shin, N. A. Pierce, *Nano Lett.* **2004**, *4*, 905–909.
[4] H. Nakao, M. Gad, S. Sugiyama, K. Otobe, T. Ohtani, *J. Am. Chem. Soc.* **2003**, *125*, 7162–7163.
[5] a) Z. Gueroui, C. Place, E. Freyssingeas, B. P. Berge, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 6005–6010; b) Y. Y. Liu, P. Y. Wang, S. X. Dou, W. C. Wang, P. Xie, H. W. Yin, X. D. Zhang, *J. Chem. Phys.* **2004**, *121*, 4302–4309.
[6] H. Yokota, J. Sunwoo, M. Sarikaya, G. van den Engh, R. Aebersold, *Anal. Chem.* **1999**, *71*, 4418–4422.
[7] a) P. Bjork, A. Herland, I. G. Scheblykin, O. Inganas, *Nano Lett.* **2005**, *5*, 1948–1953; b) D. Mijatovic, J. C. T. Eijkel, A. van den Berg, *Lab Chip* **2005**, *5*, 492–500.
[8] a) N. L. Rosi, C. A. Mirkin, *Chem. Rev.* **2005**,*105*, 1547–1562; b) L. M. Demers, D. S. Ginger, S. J. Park, Z. Li, S. W. Chung, C. A. Mirkin, *Science* **2002**, *296*, 1836–1838.
[9] G. J. Zhang, T. Tanii, T. Funatsu, I. Ohdomari, *Chem. Commun.* **2004**, *7*, 786–787.
[10] a) V. Dugas, J. Broutin, E. Souteyrand, *Langmuir* **2005**, *21*, 9130–9136; b) M. Fujita, W. Mizutani, M. Gad, H. Shigekawa, H. Tokumoto, *Ultramicroscopy* **2002**, *91*,281–285.
[11] S. A. Lange, V. Benes, D. P. Kern, J. K. Heinrich Horber, A. Bernard, *Anal. Chem.* **2004**, *76*, 1641–1647.
[12] P. Pammer, R. Schlapak, M. Sonnleitner, A. Ebner, R. Zhu, P. Hinterdorfer, O. Hoglinger, H. Schindler, S. I. Howorka, *ChemPhysChem* **2005**, *6*, 900–903.
[13] F. Matsumoto, M. Kamiyama, K. Nishio, H. Masuda, *Jpn. J. Appl. Phys.* **2005**, *44*, L355–L358.
[14] M. Cavallini, F. Biscarini, J. Gomez-Segura, D. Ruiz, J. Veciana, *Nano Lett.* **2003**, *3*,1527–1530.
[15] a) Y. Xia, G. M. Whitesides, *Angew. Chem.* **1998**, *110*, 568–594; *Angew. Chem. Int. Ed.* **1998**, *37*, 550–575; b) D. Qin, Y. Xia,

G. M. Whitesides, *Adv. Mater.* **1996**, *8*, 917 – 919; c) M. Cavallini, P. Stoliar, J. F. Moulin, M. Surin, P. Leclère, R. Lazzaroni, D. W. Breiby, J. W. Andreasen, M. M. Nielsen, P. Sonar, A. C. Grimsdale, K. Müllen, F. Biscarini, *Nano Lett.* **2005**, *5*, 2422 – 2425.
[16] M. G. Cacace, E. M. Landau, J. J. Ramsden, *Q. Rev. Biophys.* **1997**, *30*, 241 – 278.
[17] G. Reiter, *Langmuir* **1993**, *9*, 1344 – 1351.
[18] D. Beysens, C. M. Knobler, *Phys. Rev. Lett.* **1986**, *57*, 1433 – 1436.
[19] a) M. Brinkmann, F. Biscarini, C. Taliani, I. Aiello, M. Ghedini, *Phys. Rev. B* **2000**, *61*, R16339 – R16342; b) M. Brinkmann, S. Graff, F. Biscarini, *Phys. Rev. B* **2002**, *66*, 165430 – 165438; c) M. Cavallini, F. Biscarini, S. Léon, F. Zerbetto, G. Bottari, D. A. Leigh, *Science* **2003**, *299*, 531.
[20] R. Seemann, S. Herminghaus, K. Jacobs, *Phys. Rev. Lett.* **2001**, *86*, 5534 – 5537.
[21] R. Xie, A. Karim, J. F. Douglas, C. C. Han, R. A. Weiss, *Phys. Rev. Lett.* **1998**, *81*, 1251 – 1254.
[22] P. Doty, B. Bunce McGill, S. A. Rice, *Proc. Natl. Acad. Sci. USA* **1958**, 44, 432 – 438.

