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Writing with DNA and Protein Using a Nanopipet for Controlled Delivery

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There is now a great interest in manipulating biological material at the nanometer scale to produce novel structures.[1−9] The current methods are based on microcontact printing (μCP)[2,3] or microfluidic devices (μFDs).[4,5] While μCP can provide submicrometer-feature size,[3] this technique has little control over the amount of material deposited. Using μFDs it is generally difficult to obtain feature sizes smaller than several micrometers because of difficulties in device fabrication and practical problems due to channel blocking. In principle scanning probe microscopy (SPM) methods offer the possibilities of smaller feature size, and the use of dip-pen nanolithography (DPN)[6−9] was recently reported for DNA[7] and proteins.[8,9] Both, μCP and DPN use the difference in surface adsorption to transfer ink first to the stamp or tip and then to the surface. Here we present a conceptually different method for direct patterning of surfaces with biological material, which offers the advantage of fine control of the delivery potentially down to the single-molecule level.

Our method is based on a form of SPM called scanning ion-conductance microscopy (SICM)[10] that was developed to scan soft nonconducting materials by using an electrolyte-filled micropipet as a probe. The robust SICM distance control allows routine imaging of the surface of living cells.[11] It has been shown that the pipet can act as a local reservoir of reagents that can be delivered to the cell surface for functional mapping of ion channels.[12] Thus far, the SICM has only been used to write micrometer-size copper structures onto conducting surfaces.[13,14] A closely related method, scanning electrochemical microscopy, has also been used to pattern silver dots onto a gold substrate.[15] In SICM, the ion current flowing between an electrode inside the nanopipet probe and an electrode in the bath is used to control the pipet−sample distance.[16] We used pipets with inner diameters of 100−150 nm controlled at 100−150 nm above a glass surface.[17] Small quantities of biotinylated and fluorophore-labeled DNA or protein G were delivered by the pipet to the surface where they are immobilized by biotin−streptavidin binding or electrostatic interaction, see Figure 1.

The electric field in the tip region is nonuniform along the pipet axis due to the conical shape.[18] The number of molecules exiting the tip depends on a combination of electroosmotic flow, electrophoresis, and dielectrophoresis, depending on the size, charge, and polarizability of the molecules. It is therefore necessary to characterize the delivery of molecules from the pipet experimentally. We have previously studied the flow of fluorophore-labeled single-stranded DNA (ssDNA) out of the nanopipet using single-molecule fluorescence measurements at the tip of the pipet.[18] When the counter electrode was at a negative potential relative to the pipet, there was negligible flow of DNA out of the tip. On application of a positive potential, DNA flow occurred, and the flux was linear with applied potential. This gave fine control of the rate of DNA delivery by controlling the applied potential over a voltage range from 0.2 to 1.0 V.

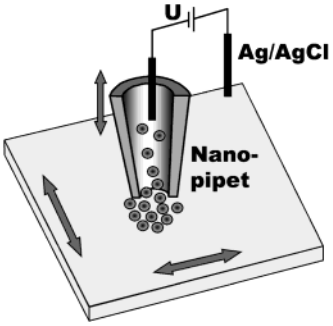

Figure 1. Schematic of the writing experiment. A voltage is applied between two Ag/AgCl electrodes, one inside the nanopipet and one inserted into the bath of ionic solution. The pipet is filled with a 100 nM solution of DNA or protein. The ion current is used as fine control of the molecule delivery as well as for the tip−surface distance control.

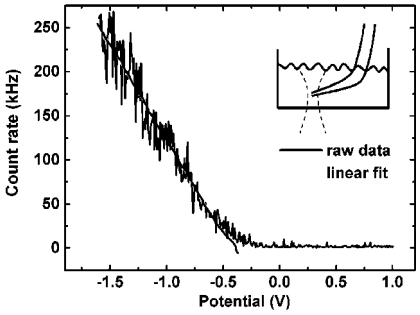

Figure 2. Fluorescence intensity of Alexa 488-labeled protein G as a function of the applied voltage, the scan speed was 65 mV/s. The inset shows the configuration with a bent pipet used in this experiment.

In the case of protein G the molecules exit the pipet on application of a negative potential, and a linear relationship between applied voltage and number of molecules delivered was obtained, see Figure 2. ssDNA[19] was deposited on a streptavidin-coated glass surface,[20] using ion conductance control of the pipet-to-sample distance. The sample piezo stage carrying the glass slide was manipulated with nanometer precision by manually changing the input voltages. In these experiments the voltage applied to the counter electrode was kept constant at 600 mV, so that the flux out of the pipet was about 4000 molecules/s. After writing a feature, the surface was retracted about 15 μm by the sample piezo stage so that the DNA exiting the tip diffuses into the open volume and dilutes rapidly.

Fluorescence of the Rhodamine Green-labeled ssDNA at 488 nm excitation was detected by scanning confocal microscopy. The detection was performed on the same instrument with 450 nm

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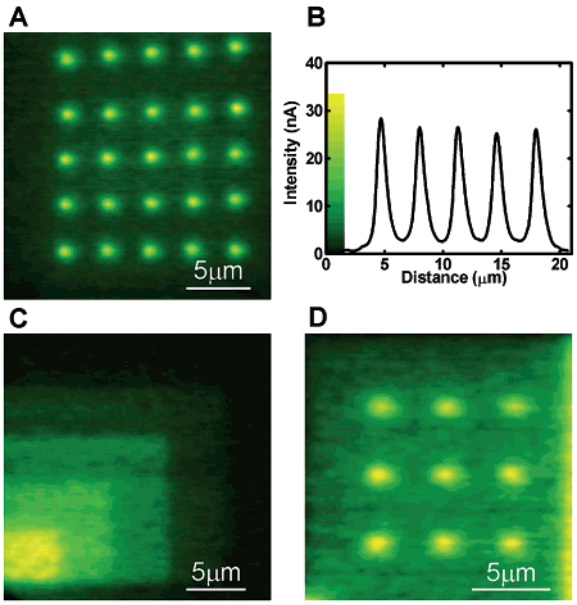

Figure 3. Fluorescence images **(**A**)** 25 dots of biotinylated DNA deposited for 10 s each onto a streptavidin-coated glass surface, the image size is 21 × 21 μm. **(**B**)** Line scan of the bottom row in Figure 3A, the fwhm is 830 ± 80 nm. **(**C**)** Squares of biotinylated DNA (4−17 μm), written one over the other to create a pattern with increasing intensities. The image size is 21 × 21 μm. **(**D**)** Dots of protein G on a positively charged glass surface, the image size is 17 × 17 μm.

optical resolution, so that the image shows a convolution of the Gaussian-shaped instrument function and the distribution of DNA molecules on the surface. Figure 3A shows an array of 25 spots, each deposited for 10 s. The measured full width at half-maximum (fwhm) is 830 ± 80 nm, and the intensity varies only ±6%. Because of diffusion out of the pipet and on the surface, the intensity does not decrease to zero between the spots. Hence, a wider pitch of about 6 μm would be necessary to eliminate any overlap between the spots, if required. By performing quantitative fluorescence measurements on DNA spots of known surface densities we estimate the number of molecules in one spot to be 46000 ± 12000. The number of molecules flowing out of the pipet can vary for different pipets, in the case of DNA by a factor of 2. [21] On the basis of our single-molecule counting experiment,[18] we estimate 20000−80000 molecules during 10 s deposition time. The efficiency of attaching the molecules to the surface is therefore estimated to be at least 40%. The remainder of the molecules diffuse into the open volume.

Using computer control to scan the stage, more complex patterns can be produced. In Figure 3B squares were written one over another, so that areas of different intensity are produced. This demonstrates the possibility of not only the formation of patterns but also of writing in "gray scale".

On application of −500 mV to the electrode in the bath, nine spots of protein G were deposited onto a positively charged glass surface, see Figure 3D. The protein G was immobilized by electrostatic interaction, with a measured feature size of 1.3 μm.

Since the pipet is operating in solution, the feature size should depend on the distance the pipet is held from the surface and on diffusion. In a simple model, the feature size can be estimated using the steady-state concentration profile derived for ultra-microelectrodes in the diffusion-limited case.[22,23] The calculation for a pipet of 100 nm diameter held at 120 nm distance from the surface leads to a fwhm of 450 nm for the concentration profile. After deconvolution with the instrument function, the observed feature size is a factor of 1.6 larger in the case of DNA on streptavidin-coated glass and a factor of 3 in the case of protein G. This may be due to 2D diffusion of the biomolecules on the surface and, in the case of DNA, the density of streptavidin sites on the glass surface.

The method presented here is based on available scanning probe and micropipet technology. It offers the advantage of operating under physiological conditions so that it should be straightforwardly applicable to other biological molecules such as enzymes and antibodies. It allows fine control of the density of the deposited molecules on the surface potentially down to the single-molecule level, as single-molecule delivery from the nanopipet has already been demonstrated.[18] In addition subsequent assays can be performed on surface-attached molecules by local application of one or more reagents from the pipet.

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Supporting Information Available: Experimental details and additional images (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Whitesides, G. M.; Ostuni, E.; Takayama, S.; Jiang, X.; Ingber D. E. *Annu. Rev. Biomed. Eng.* **2001**, *3*, 335−373.
(2) James, C. D.; Davis, R. C.; Kam, L.; Craighhead, H. G.; Isaacson, M.; Turner, J. N.; Shain, W. *Langmuir* **1998**, *14*, 741−744.
(3) Bernard, A.; Delamarche, E.; Schmid, H.; Michel, B.; Bosshard, H. R.; Biebuyck, H. *Langmuir* **1998**, *14*, 2225−2229.
(4) Delamarche, E.; Bernard, A.; Schmid, H.; Michel, B.; Biebuyck, H. *Science* **1997**, *276*, 779−781.
(5) Bernard, A.; Michel, B.; Delamarche, E. *Anal. Chem.* **2001**, *73*, 8−12.
(6) Piner, R. D.; Zhu, J.; Xu; F.; Hong, S.; Mirkin, C. A. *Science* **1999**, *283*, 661−663.
(7) Mirkin C. A. *MRS Bull.* **2000**, *25*, 43.
(8) Wilson, D. L.; Martin, R,; Hong, S.; Cronin-Golomb, M.; Mirkin, C. A.; Kaplan, D. L. *Proc. Natl. Acad. Sci.* U.S.A. **2001**, *98*, 13660−13644.
(9) Lee, K. B.; Park, S. J.; Mirkin, C. A.; Smith, J. C.; Mrksich, M. *Science* **2002**, *295*, 1702−1705.
(10) Hansma, P. K.; Drake, B.; Marti, O.; Gould, S. A. C.; Prater, C. B. *Science* **1989**, *243*, 641−643.
(11) Korchev, Y. E.; Bashford, C. L.; Milovanovic, M.; Vodyanoy, I.; Lab, M. J. *Biophys. J.* **1997**, *73*, 653−658.
(12) Korchev, Y. E.; Negulyaev, Y. A.; Edwards, C. R. E.; Vodyanoy, I.; Lab, M. J. *Nat. Cell Biol.* **2000**, *2*, 616−619.
(13) Zhang, H.; Wu, L.; Huang, F. *J. Vac. Sci. Technol., B* **1999**, *17*, 269−272.
(14) Müller, A. D.; Müller, F.; Hietschold, M. *Thin Solid Films* **2000** *366*, 32−36.
(15) Yatziv, Y.; Turyan, I.; Mandler, D., *J. Am. Chem. Soc.* **2002** *124*, 5618−5619
(16) Shevchuk, A. I.; Gorelik, J.; Harding, S. E.; Lab, M. J.; Klenerman, D.; Korchev, Y. E. *Biophys. J.* **2001**, *81*, 1759−1764.
(17) The pipet diameter was determined by SEM images, see Supporting Information, Figure S1. The pipet−sample distance was determined by approach curves, see Supporting Information, Figure S2.
(18) Ying, L. M.; Bruckbauer, A.; Rothery, A. M.; Korchev, Y. E.; Klenerman, D. *Anal. Chem.* **2002**, *74*, 1380−1385.
(19) The sequence of the DNA is 5′-CTATGCAGCCATTGTAGTCC-3′. The 3′-end is modified by Rhodamine Green (Molecular Probes, Leiden, The Netherlands), and the 5′-end is modified with biotin.
(20) Glass-bottomed dishes (WillCo, Wells B.V., The Netherlands) were coated with streptavidin by BioTeZ Berlin-Buch GmbH, Germany.
(21) In the case of protein delivery, preliminary results show that coating the pipet tip with poly(ethylene glycol) (PEG) silane improves the reproducibility between pipets.
(22) Bond, A. M.; Oldham K. B.; Zoski, C. G. *J. Electroanal. Chem.* **1988**, *245*, 71−104.
(23) The presence of the glass surface near the tip and the pipet wall thickness are neglected for simplicity.

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