Ordered stretching of single molecules of deoxyribose nucleic acid between microfabricated polystyrene lines

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A technique for creating arrays of parallel, stretched single molecules of deoxyribose nucleic acid (DNA) on an arbitrary substrate for high-resolution scanning-probe imaging is discussed. The technique consists of lithographically patterning polystyrene lines on a substrate which then provide attachment sites for the ends of individual DNA molecules. Molecular combing is performed to stretch DNA from one polystyrene line to the other. Scanning-tunneling and atomic-force microscope images of single molecules of bacteriophage-lambda DNA are shown to demonstrate the advantages of this technique. Several applications, from high-resolution genomics to molecular electronics, are discussed. © *2001 American Institute of Physics.* [DOI: 10.1063/1.1365099]

Because of the recent drive towards realizing truly molecular-scaled devices, there is a great need to develop techniques which can control the placement of single molecules onto micro- or nanofabricated devices. Here, we describe a technique we have developed based on ''molecular combing.''[1–3] This technique aligns and attaches single molecules of deoxyribose nucleic acid (DNA) to site-specific areas of an arbitrary substrate without the need for special chemical modification of the DNA molecules themselves[4] or modified gold nanoparticles or latex beads.[5] It is easy to employ, highly parallel, and results in high yield. It is, therefore, suitable not only for research but also for industrial applications as well.

Used in molecular biology for high-resolution genomic studies,[2] molecular combing is a technique which stretches and randomly positions double-stranded DNA onto a surface-specific substrate such as glass or silane-treated substrates. As we demonstrate below, the utility of molecular combing can be greatly expanded when it is used in combination with current lithographic techniques: single molecules of DNA can be stretched, patterned, and directed site specifically onto an arbitrary surface such as gold or bare silicon. This, consequently, leads to a number of important potential applications in a diverse set of fields ranging from molecular electronics to biotechnology. For example, patterned DNA on a substrate can serve as templates for wires and for two- and three-dimensional nanoscale devices. In another example, stretching and attaching DNA to specific areas of a substrate can assist in high-resolution genomic studies in terms of mapping samples onto the substrate. Here, we focus on stretching single molecules of bacteriophage-lambda (phage-λ) DNA onto conducting substrates for high-resolution scanning-tunneling microscopy (STM) and atomic-force microscopy (AFM) studies. High-resolution STM and AFM studies of long DNA molecules are difficult to achieve because the DNA often coils, aggregates, and moves under the scanning-probe tip while resting on a substrate surface.[6–10] We will show that the technique we have developed greatly facilitates such studies as the DNA is stretched, isolated, and rigidly attached to the substrate surface. We show both STM and AFM images of stretched, phage-λ DNA that clearly demonstrate the advantages of our technique.

Molecular combing begins by coating a silicon substrate with a 300-nm-thick layer of polystyrene. The substrate is then dipped into a 50 pM solution of phage-λ DNA in 50 mM 4-morpholine–ethanesulfonic acid (MES) buffer solution (pH=5.65).[11] As illustrated in Fig. 1, the ends of a DNA molecule preferentially bind to the polystyrene surface. While the exact binding mechanism is not clearly understood, it is presumably due either to an electrophilic addition of weak acids to alkenes,[1–3] hydrophobic interaction,[12] or to induced dipole–dipole interactions between the substrate surface and the DNA molecule. Because the process is not instantaneous, the prepared substrate needs to remain in solution for a short incubation time of ~1 min so that one end of the DNA may bind to the surface. The substrate is then retracted from the buffer/DNA solution at a constant speed of 30 μ/s using a syringe pump. The meniscus force (~100 pN) is strong enough to extend, or stretch, the DNA molecule but far too weak to break the bond between the DNA and the polystyrene surface. Once the DNA molecule leaves the buffer solution, its remaining free end binds to the substrate surface. As shown in the AFM image in Fig. 2, this entire process leads to uniformly stretched, parallel arrays of DNA molecules on the substrate surface.

Interestingly, the density of molecules stretched across a surface does not increase significantly with increasing concentration of DNA solution; rather, it is dependent on the

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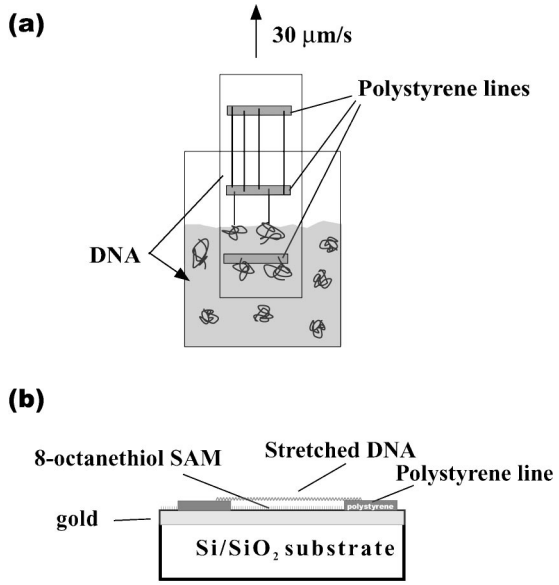

FIG. 1. (a) Illustration of the molecular combing process. The substrate is dipped into a buffer solution containing DNA and then retracted at a constant speed of 30 μm/s. The DNA molecules bind to the polystyrene lines and are stretched by a meniscus force. (b) Schematic picture of a prepared sample consisting of a DNA molecule stretched between a set of patterned polystyrene lines.

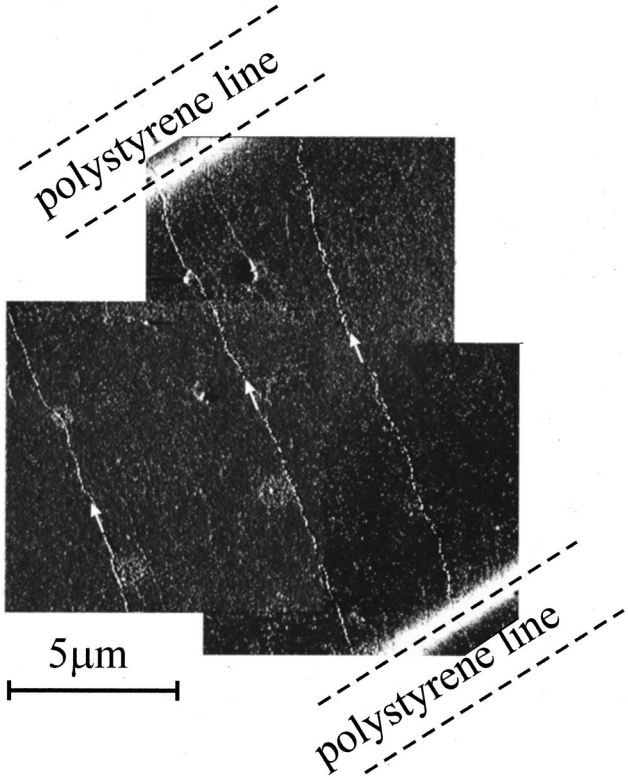

FIG. 3. TM amplitude AFM image showing bacteriophage-λ DNA stretched between patterned polystyrene lines. White arrows point to the DNA. The distance between polystyrene lines is 17.5 μm and the height of the lines is 300 nm.

radius of gyration R_G of the DNA molecule in solution. Here, $R_G \propto L^{3/5}$, where L is the crystallographic length of the molecule, and $2R_G$ is the average size of the coiled DNA molecule in solution. For phage-λ DNA, $R_G = 0.73$ μm.[13] The fact that we find the distance separating stretched phage-λ DNA molecules to be close to this R_G value (see Fig. 2) supports this view. Thus, to increase the density of stretched DNA, it is necessary for us to repeat the molecular combing procedure outlined above a number of times (the already-combed molecules do not unbind upon rehydration).

The binding of DNA can be localized to specific substrate areas using lithographically patterned polystyrene. As had been previously demonstrated,[14,15] polystyrene is an effective negative electron-beam (e-beam) resist: e-beam irradiation induces cross links between polystyrene chains, thereby making these particular chains insoluble to solvents such as xylene. Because DNA binds equally to cross linked and uncross-linked polystyrene, we can, consequently, e-beam polystyrene patterns on a substrate and stretch DNA on or between these patterns.

To demonstrate our ability to stretch DNA site specifically and perform subsequent STM measurements, we fabricated a grid of 2-μm-wide polystyrene lines (lattice constant $a = 17.5$ μm) on a conducting substrate using standard e-beam lithography. The patterned substrate was dipped into a 1 mM ethanolic solution of octanethiol. Octanethiol forms an ordered self-assembled monolayer (SAM) on a gold surface,[16] which (1) makes the surface hydrophobic, thus matching the surface tension between the gold and the polystyrene; and (2) provides a good tunnel barrier for STM studies. After an overnight incubation in octanethiol to allow sufficient time for the SAM to form, the substrate was carefully rinsed with ethanol and molecular combing was immediately performed. Fluorescent imaging of samples combed with phage-λ DNA [dyed with the fluorescent probe, YOYO-1 (Ref. 17)] showed that we achieved a DNA density of ~1 molecule/μm stretched across the grid of polystyrene lines. Figure 3 is an AFM image of one of these samples. It should be noted that in this geometry we never observed loops of DNA molecules bound to a single polystyrene line. This is presumably due to the weak interaction between the DNA molecule and the SAM, which keeps the DNA stretched across the substrate surface.

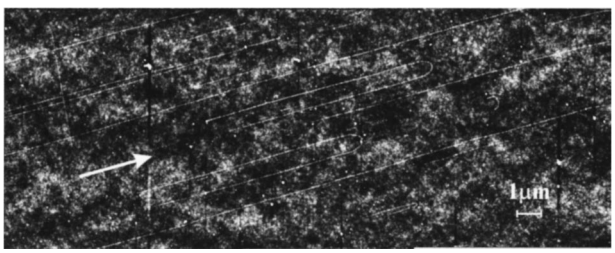

FIG. 2. Array of stretched bacteriophage-λ DNA on a gold surface coated with a thin polystyrene layer. The substrate was dipped in a solution of 50 pM bacteriophage-λ DNA solution and retracted at a constant speed of 30 μ/s using a syringe pump. The image shown is an AFM TM amplitude image; the size is 9×23 μm^2. The white arrow indicates the direction of stretching.

We have performed high-resolution scanning-probe measurements on similarly prepared samples using a Nanoscope multimode STM/AFM. Figure 4 shows a STM image, taken at room temperature, of a DNA molecule stretched between a set of polystyrene lines. The contrast of the single molecule in the STM image is inverted (the molecule is visible as a dark line). This phenomenon has been observed in other STM measurements of DNA molecules deposited on metal surfaces[9,10,18,19] and is related to either the buffer spe-

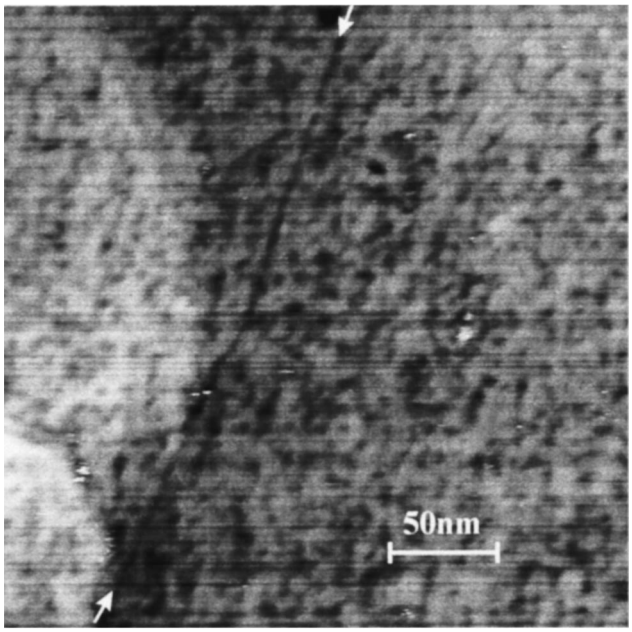

FIG. 4. Room-temperature STM image of a DNA molecule stretched on a single gold grain between a set of patterned polystyrene lines. The bias voltage was 1 V and the current, 5 pA; white arrows point to the DNA. The structure in the background corresponds to the domains of the octanethiol self-assembled monolayer. The gold has been flame annealed until the mean grain size was in the range of 300–500 nm.

cies co-adsorbed around the DNA molecules or the hybridization effects between the DNA and the underlying metal. The image shown in Fig. 4 nonetheless demonstrates the advantages of our technique. Because we were able to position the DNA site specifically on our substrate, we could easily align the scanning-probe tip to the molecules. Exhaustive ''searching'' for molecules is thus not necessary. Since the DNA was stretched and fixed in position, we could image the molecule with greater clarity than if we had just deposited the DNA on the surface. Base-pair resolution, however, is difficult as we had used double-stranded DNA. The phosphate backbone of the double-stranded DNA hides the individual bases.

In conclusion, we have presented a technique for stretching and positioning DNA site specifically onto an arbitrary surface. This technique consists of molecular combing DNA between lithographically patterned polystyrene. It can potentially place DNA molecules on a substrate with nanometer precision, given that we can e-beam nanometer-sized patterns of polystyrene on a substrate. As we have shown, we have prepared stretched, immobile, and well-positioned DNA onto conducting surfaces for high-resolution scanning-probe studies: the room-temperature STM and AFM images we have obtained are of high quality. The DNA we have probed have all been double stranded, and consequently, the phosphate backbone of the double helix prevents the imaging of individual bases. While double-stranded DNA is necessary for the selective and high-affinity binding to polystyrene, one can perform standard biotechnology techniques[20] to insert a single-stranded piece of DNA in the middle of a double-stranded one to expose neatly aligned bases for imaging and possible genomic identification.

While we have focused on high-resolution scanning-probe studies as an application to our technique, there are a great many other potential applications which deserve attention here. Because it can be patterned onto an arbitrary substrate, DNA can serve as a template for wires and nanoscaled devices.[21,22] Recently, Wooley *et al.*[23] have demonstrated the possibility of detecting polymorphic sites and of determining directly haplotypes in appropriately labeled DNA fragments using a scanning-probe tip. Our technique could prepare ordered stretched DNA for similar scanning-probe studies, and in addition, provide the possibility of automation since the DNA molecules can be neatly aligned in arrays. Equally intriguing, our technique could prepare groups of differently labeled DNA–all mapped onto different locations of a substrate, much like that of current DNA microarrays—for systematic studies of population-based genetic diseases and genomic screening. Thus, molecular combing in combination with microlithography has potential applicability in a number of diverse areas which can simplify the automate sample preparation and measurement.

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