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Patterning DNA on μm scale on mica

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Abstract

Double-stranded DNA molecules were patterned by selective adsorption to aminosilane patterns on mica surfaces. Line patterns with 10 μm spacing were made by photolithography and transferred to a polymer stamp. The stamp was then used for applying aminosilane molecules by microcontact-printing technique on mica substrates. We applied DNA in Tris-EDTA (TE) buffer solution on the patterned substrate, and incubated it for 5 min at room temperature. The sample was then rinsed with pure water, and dried with nitrogen gas. Tapping mode force microscopy showed that DNA was adsorbed selectively on the aminosilanized parts of the mica substrate. We also tried to bridge two aluminum electrodes with DNA using AC electrophoresis.

Keywords: DNA; Aminosilane; Microcontact printing; Atomic force microscopy

1. Introduction

Microarrays of complementary DNA have been successfully applied for monitoring gene expressions. High-density DNA arrays were fabricated by 'light-directed' oligonucleotide synthesis [1], and patterns as small as 20 μm were utilized [2]. Another type of microarrays is fabricated without photomasking process, but by direct application of small droplets (~0.005 μl) of polymerase chain reaction (PCR) products, which spread over 100 μm and resulted in a larger pattern [3,4]. Confining DNA within 10 μm on a substrate could help fabricating higher density microarrays with the latter method.

In addition to applications like DNA chips and computing, DNA is expected to contribute in future molecular electronics as a molecular wire. DNA network structures [5–7] or nanoparticle-DNA complex may present new electronic properties, e.g. combined effects of quantum interference and single electron tunneling. For assembling DNA with other electronic components, precise techniques for positioning DNA and/or DNA complexes will be needed. Since DNA was found to adsorb selectively on aminosilane films [6,8,9], we utilized such property to pattern it. This was

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done by creating aminosilane patterns on mica surfaces by the microcontact printing (μCP) technique [10,11].

Most of the μCP reports used thiols on gold substrates, while there have been relatively few reports [12–14] applying the technique to silane-oxide, i.e., molecules on insulator system, which will be more practical to combine with semiconductor devices. One form of future molecular devices will be fabricated using electrodes with nanometer-scale insulative spacings where functional molecules are to be immobilized to act as nanodevices. Such molecules are supposed to be selectively adsorbed in the gap, aligned between the electrodes by, e.g., electric fields, and connected to the surfaces of the electrodes. We will demonstrate an example of this type of approach in the following section. It was in 2000 that Finnie et al. studied the formation mechanism and quality of silane self-assembled monolayers (SAMs) by μCP on Si oxide [15]. Furthermore, Finnie et al. and Wang et al. showed that the octadecyltrichlorosilane (OTS) and docosyltrichlorosilane (DTS) patterns transferred to silicon surfaces worked as resists against KOH etching [15,16]. Huang et al. utilized 'selective dewetting' of diazonaphthoquinone novolak polymer (photoresist) for patterned growth of carbon nanotubes [14].

Most of silane μCP studies used alkyltrichlorosilanes such as OTS and DTS, in contrast to a few reports in which aminosilanes were used for silanization [17]. To step forward towards the goal of molecular scale construction, we believe that direct chemical reaction with the patterned SAM films will be necessary. In general, for constructing complex molecular structures on target surfaces, it will be easier to build them from smaller soluble molecules using surface chemical reactions than to synthesize complex large molecules separately and adsorb them on the surface: This is because such large molecules are often insoluble and hard to be handled. In this work, however, we patterned double-stranded DNA molecules using aminosilane films on mica, since techniques for handling DNA are well-known. With the μCP technique, we could readily confirm the selectivity of DNA adsorption on bare and aminosilanized areas on mica surfaces.

We further tried to control molecular orientation using an electric field between comb shaped aluminum electrodes. Stretched DNA molecules were observed in the silanized areas between the electrodes.

2. Materials and methods

Photoresist patterns of 5 and 10 μm line-and-space were prepared by photolithography on a silicon substrate which was used as a master for the stamp. Patterned poly(dimethylsiloxane) (PDMS) stamps were made by mixing silicone elastomer and curing agent (Dow Corning Co., SYLGARD184), and pouring the mixture in a plastic disposable container with the silicon master at the bottom. The container was then evacuated to remove the bubbles, and heated in an oven at 60°C for 12 h in air. Then we took the hardened polymer out from the container, removed the master, and cut the elastomer in a suitable shape around the stamp. We applied 1 mM hexane solution of 3-(2-aminoethylamino)propyltriethoxysilane (aminosilane) directly to the PDMS stamp in a globe box filled with nitrogen gas.

Then aminosilane molecules were microcontact-printed on mica substrates for 20–30 min (Fig. 1(a)). We applied 175 μg/ml λDNA (48.5 kbp) on the patterned substrate (Fig. 1(b)), washed with pure water, and dried under nitrogen gas (Fig. 1(c)).

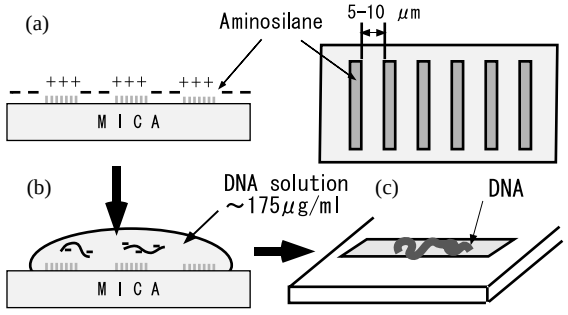

Fig. 1. μCP aminosilane and DNA patterning scheme.

3. Results and discussion

Tapping mode atomic force microscopy (AFM) showed a high selectivity of DNA adsorption on the aminosilanized patterns compared to bare mica as shown in Fig. 2.

The observed high selectivity may be explained by electrostatic interaction between DNA and substrate. DNA molecules are negatively charged due to the negative charges of the phosphate backbone in the buffer solution. The surface of cleaved mica is also negatively charged, while the aminosilanized surface is supposed to exhibit positive charge [5] (Fig. 1(a)), and attracts DNAs. DNA molecules are mobile on untreated mica, and can be easily washed off. Fig. 2 shows DNA molecules that were strongly adsorbed to aminosilane and withstood washing and drying processes.

As Pompe et al. called the 'elevated rim' structure and explained by the capillary condensation of excess solution in their article [13], the boundaries of the microcontact-printed pattern of aminosilane tend to protrude similarly (Figs. 2(a) and (e)). Aminosilane molecules sometimes seem to polymerize and form protruded structures as shown in Fig. 2(c). The protrusions can be bundled or globular form of DNAs [18]. In this case, uniform films may not be formed, but around those structures, aminosilane molecules are present, and even the aggregated structures seem to adsorb DNA molecules.

In Figs. 2(a) and (b), DNA molecules are seen outside the elevated rims about 500 nm (e.g., the white arrow in Fig. 2(b)), probably due to the spillover of aminosilanes from the edge of the stamp. Another patterning problem can be observed in the form of tilted lines over the pattern (Fig. 2(a)). This may be formed as a result of the displacement of the stamp during contacting mica surface. Careful maneuver or controllable device is required to improve patterning precision down to 100 nm.

In addition to the precise positioning, techniques for stretching DNA have been extensively studied [19,20]. Recently, we succeeded in orienting DNA molecules by tethering them from one end on mica surfaces through a crosslinker and

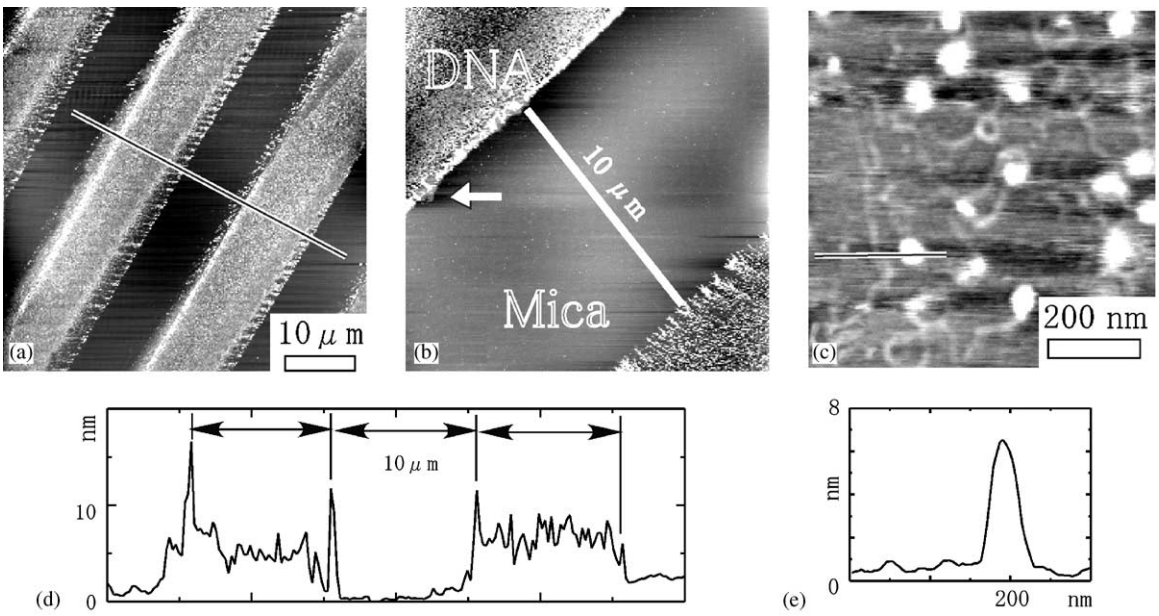

Fig. 2. DNA selectively adsorbed on μCP aminosilane. (a) and (b) Height of the DNA part measures about 3 nm, indicating bundle formation. The white arrow in (b) indicates a position where the wall along the boundary is broken and some DNAs are adsorbed outside the patterned area. (c) Height of the DNA measures about 0.5 nm, which is typical for AFM observation of single DNAs [6,24]. About the protrusions, see the text. (d), (e) Crosssectional profiles along the lines in AFM images (a) and (c), respectively.

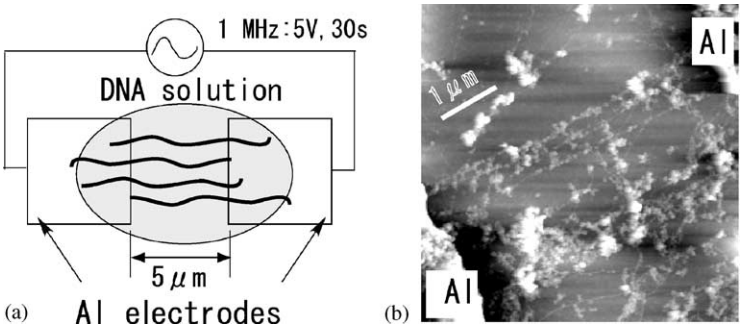

Fig. 3. DNAs bridging Al electrodes 5 μm apart. In addition to the stretched DNA strands, there remain some unspecific structures, which may be by-products due to some electrochemical reaction.

stretching them by shear force [20]. In this work, we tried to bridge two electrodes with DNA using AC electrophoresis (Fig. 3(a)). We used interdigitated electrodes with a spacing of 5 μm which were fabricated on mica using the lift-off process. After aminosilanizing the mica substrate, we covered the comb electrode with DNA solution, and applied an AC voltage of 5 V at 1 MHz which produces the same electric field as reported previously by Washizu et al. [21,22] for 30 s.

After drying the buffer in air at room temperature, large structures (most probably, salt crystals) appeared, which seemed to cover DNA. On washing the sample with Milli-Q water, DNA could be observed bridging the two electrodes (Fig. 3(b)). Even after washing, the surface was partially covered with unspecific structures, which could not be removed by the rinsing. We speculate that they could be by-products due to some electrochemical reaction.

DNA stretched by electric field was also observed using AFM by Ueda et al. [23]. In their experiment, they immobilize DNA molecules on the slope of Al electrodes. Our result shows that the DNA can be also immobilized firmly on an insulative gap area, e.g., a silanized mica surface, with the orientation parallel to the field.

4. Conclusion

We applied microcontact printing technique to pattern organic thin films on mica surfaces. 10 μm patterns made by photolithography were transferred to a silicone rubber stamp, and then aminosilane molecules were microcontact-printed on mica substrates. We applied DNA in TE buffer solution on the patterned substrates, and found that the DNA was adsorbed selectively on the aminosilanized areas with a precision of about 500 nm. We could orient DNA molecules by applying an electric field. Stretched molecules were immobilized on the aminosilanized mica substrate. Some DNA molecules remained firmly attached to the silanized areas after rinsing the sample with Milli-Q water.

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