Inkjet Deposition of Alkanethiolate Monolayers and DNA Oligonucleotides on Gold: Evaluation of Spot Uniformity by Wet Etching

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Inkjet printing allows localized, contact-free deposition of liquids onto arbitrary substrates. In this article we demonstrate the fast formation of high-quality self-assembled monolayers (SAMs) on gold surfaces. Using a selective etch process, we verify the uniformity of the deposited spots. A direct comparison with microcontact-printed SAMs on Au revealed similar resist quality as inkjet-deposited alkanethiolate SAMs. Likewise, inkjet printing of thiol-functionalized and non-thiolated single-stranded DNA oligomers formed molecular layers protecting Au from etchants. For all compounds used, we achieved etched patterns that were homogeneous and free of defects. These results indicate that an inkjet is a convenient tool for surface functionalization and the direct writing of molecular films and resists.

1. Introduction

Self-assembled monolayers (SAMs) are well-defined organic layers that can be chemisorbed onto various noble metal or oxide surfaces to tune surface properties such as wettability, repulsion of chemicals, or attachment of specific molecules or particles. The classic example is SAMs of alkanethiols on Au.[1,2] Densely packed monolayers are achieved by immersing a surface into millimolar ethanolic solutions for a few minutes as well as by microcontact printing (μCP) with contact times of a few seconds.[3−5] With inkjet deposition, the time provided for monolayer formation is significantly shorter because tiny sub-nanoliter droplets of ethanolic solutions delivered to the substrates typically evaporate within less than 1 s. In general, the evaporation of droplets on surfaces creates quite inhomogeneous drying patterns, like a dried spot of coffee on a tabletop.[6] It is consequently not obvious whether inkjet deposition can produce monolayers comparable to those obtained from established methods. Pardo et al.[7] recently demonstrated inkjet spotting of millimolar thiol solutions. These authors visualized the patterns using condensation patterns and measured the contact angles, but only over millimeter-scale areas. Contact angle measurements are difficult to apply to sub-millimeter regions and provide information only on discrete locations of the surface. So far, the quality of inkjet-deposited monolayers has not yet been investigated at microscopic dimensions.

Similar issues regarding the quality of patterns arise when the inkjet method is used to deposit thiolated oligonucleotides from aqueous buffer solutions onto Au for DNA microarrays. Inkjet depositon has been found suitable for the fabrication of DNA microarrays,[8] even for in situ DNA synthesis on glass substrates.[9] However, gold substrates are preferred for some label-free detection systems such as surface plasmon resonance[10] or cantilever sensors.[11] DNA oligomers have a significantly higher molecular weight than short alkanethiol compounds. Hence they are less mobile and require longer reaction times to chemisorb on the gold surface with their reactive thiol group and to assemble into a dense monolayer. Under typical laboratory conditions, the droplets deposited by an inkjet evaporated from the substrate within a few seconds. Can this leave sufficient time for the adsorption of a complete monolayer? The application to DNA microarrays or cantilever sensors demands simple tests to prove the quality of monolayer deposition. Standard surface characterization methods applied to DNA monolayers on gold are ellipsometry, X-ray photoelectron spectroscopy and radioactive labeling,[12,13] fluorescence labeling,[14] electrochemical investigations,[15] neutron reflectivity,[16] surface plasmon resonance,[10,17] quartz crystal microbalance,[13]

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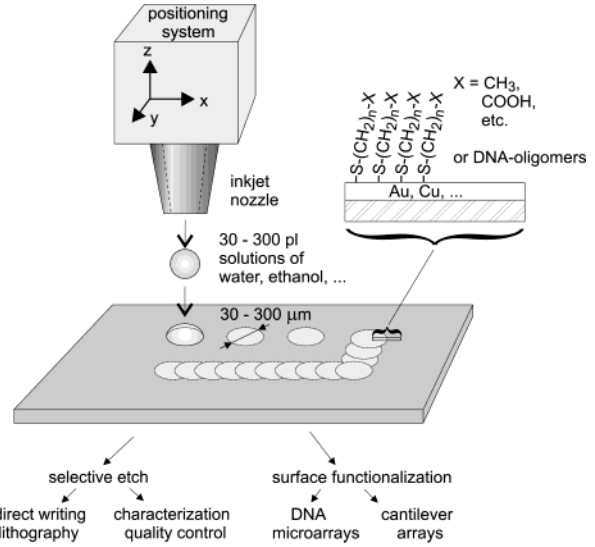

Figure 1. Application of an inkjet for local deposition of functional monolayers or resists.

capacitance measurements,[18] and atomic force microscopy.[19,20] But most of these methods do not provide satisfactory spatial resolution and are quite involved.

An indirect but very efficient method to verify the presence of a densely packed monolayer takes advantage of the ability of a SAM to inhibit the access of certain chemical species to the substrate. A selective etch of the substrate where it is not protected by a monolayer is often exploited by applications of μCP.[3] Wet etching translates the local presence of a monolayer into a Au pattern of high contrast and resolution.[5,21,22] The samples are analyzed by optical microscopy or, when higher resolution is required, by scanning electron microscopy (SEM) inspection.

In this article we apply the inkjet method to create SAMs of alkanethiols and monolayers of DNA oligonucleotides, as shown in Figure 1. We focus on a selective etch method to verify the uniformity of the deposits down to a microscopic level.

2. Materials and Methods

2.1. Solutions for Coating. All chemicals were of reagent grade or better and used as received unless indicated otherwise. Purified octadecanethiol (ODT) originated from Robinson Brothers Ltd. (West Bromwich, U.K.). Hexadecanethiol (HDT) (Fluka, Buchs, Switzerland) was purified as described in ref 5. Mercaptohexadecanoic acid (MHA) (Aldrich, Buchs, Switzerland) was dissolved in dichloromethane, and the solution was filtered and dried. Mercaptoundecanoic acid (MUA) was synthesized as described in ref 23. Thiol solutions were prepared in ethanol of puriss p.a. grade (Fluka) and stored in dark bottles.

Single-stranded DNA (ssDNA) oligonucleotides were obtained from Microsynth (Balgach, Switzerland). A thiol modification with a 5′ $HS(CH_2)_6$ linker enables a strong chemical bond to gold surfaces. A thiolated 20-mer ssDNA and both thiolated and nonthiolated 30-mers of PAGE grade were used as received. A 12-mer of HPLC grade was dissolved in water and extracted by three cycles of ethyl acetate washing followed by centrifugation and phase separation to remove thiol compounds not linked to DNA. 40 μM ssDNA sample solutions were prepared in 50 mM triethylammonium acetate (TEAA) buffer (Fluka) or deionized (DI) water.

2.2. Inkjet Deposition. We used a MD-P-705-L inkjet dispensing system (Microdrop, Norderstedt, Germany) equipped with a three-axis micropositioning system of 10 μm accuracy and piezo-driven autopipets, AD-K-501, with 70 μm nozzle diameters. A stroboscopic camera system allowed visual monitoring to adjust piezo voltages and pulse durations for reliable droplet ejection and to avoid satellite drops. Single droplets with diameters in air of 60−80 μm corresponding to volumes of 0.1−0.3 nL were ejected on demand. The vertical separation between the nozzle and the substrate was typically 0.5 mm.

2.3. Substrates. Standard Si wafers (Siltronix, Geneva, Switzerland) were coated with 1 nm Ti (99.99%, Johnson Matthey) followed by 20 nm Au (99.999%, Goodfellow) using an Edwards FL400 e-beam evaporator operated at a base pressure of $<10^{-5}$ mbar at evaporation rates of 0.03−0.04 nm/s. The fresh substrates were used within 2 days of Au deposition.

2.4. Microcontact Printing and Etching. Elastomeric stamps for μCP were made of poly(dimethylsiloxane) (PDMS). We used Sylgard 184 (Dow Corning, Midland, MI) molded on a lithographically generated photoresist pattern, as described in ref 24. For inking, the patterned side of the stamp was covered with 1 mM ODT solution for 30 s and then carefully blown dry with a stream of nitrogen. The stamp was placed manually onto the wafer substrate by applying gentle pressure to achieve conformal contact. It was left on the substrate for 1 min. A complete SAM of ODT can be created under these conditions in the regions of contact.[5,22] An etch bath, developed recently to etch microcontact-printed Au with high selectivity,[22] was composed of 20 mM $Fe(NO_3)_3 \cdot 9H_2O$ (Fluka) and 30 mM thiourea (Fluka) in DI water. After printing, the substrate was put into the etch bath until the nonprinted Au was completely removed. In a well-stirred bath, the etch times were 3−6 min for 20 nm of Au at room temperature. Samples were rinsed with DI water and ethanol and inspected with optical microscopy or SEM (Leo 1550, LEO, Oberkochen, Germany).

3. Results and Discussion

3.1. Alkanethiols. To characterize the quality of inkjet-deposited monolayers, we created an array of single droplets of ODT solution with a pitch of 1 mm. Upon deposition on the substrate, the droplets evaporated in less than 1 s. As a reference, a regular pattern of ODT monolayer was subsequently microcontact printed over the same sample, as shown in Figure 2a. The sample was then rinsed with ethanol to remove excess ODT. The selective etch bath clearly developed the printed pattern as well as the spots created by the inkjet. In the case of incomplete monolayers the etch partially attacked the gold film. The etched sample was inspected by SEM; see Figure 2b−d. As expected, the control pattern was developed by completely removing the Au versus an intact Au film of the printed pattern. Likewise, the spots of about 230 μm diameter deposited by the inkjet were uniformly protected. A complete Au film without defect pits was preserved, which means that both the printed and the spotted monolayers are of high quality and free of defects (Figure 2d). The round spots were decorated by features resembling comet tails, as seen in Figure 2b,c. This has been caused by excess ODT being washed off to the side when the sample was rinsed or exposed to a flow in the etch bath. Experiments carried out with inkjet-spotted MUA, MHA, and HDT also produce continuous films with etched patterns free of defects.

We believe that the evaporation of ethanol from the deposited droplet significantly accelerates the process of SAM formation, as it increasingly concentrates the thiol molecules. In μCP the formation of a SAM is governed by

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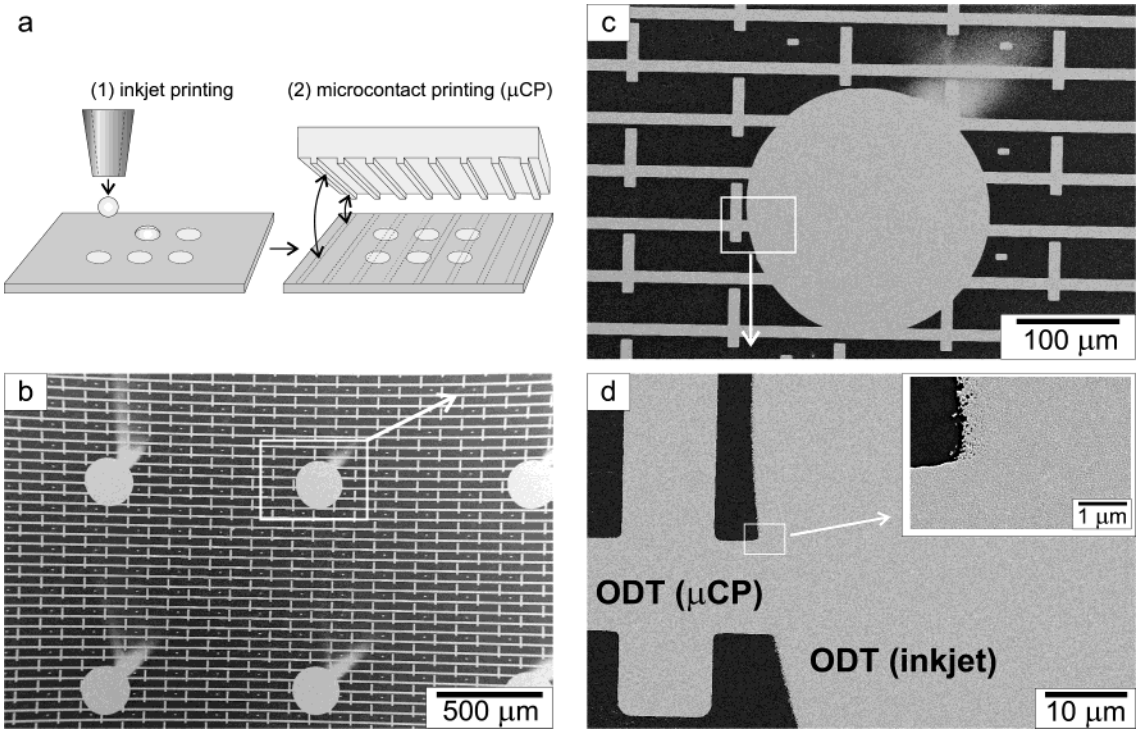

Figure 2. Gold patterns were generated as illustrated in (a) by inkjet printing (1) of ODT followed by microcontact printing (2) of ODT and subsequent selective etch. The SEM micrograph (b) shows the overview of an array of spots deposited by inkjet and the microcontact-printed reference pattern. The SAM patterns preserved the gold layer (bright areas) whereas the etch completely removed the gold from the Si substrate on nonprinted zones (dark regions). Excess ODT that had been present on the spots was washed off to the side, leaving traces that resemble comet tails. (c) No difference of quality is noticeable between the spotted and the printed SAM. (d) The high magnification views prove the absence of defects.

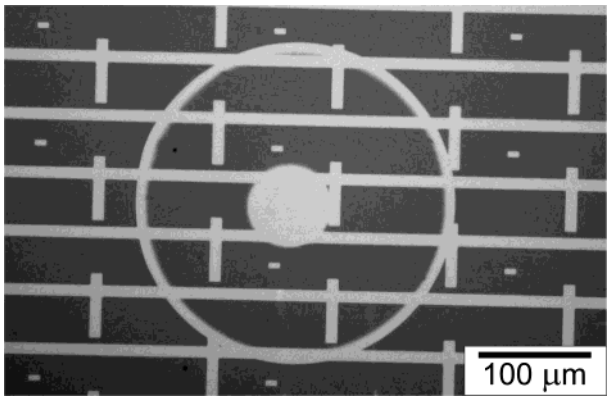

Figure 3. Optical micrograph equivalent to Figure 2b but where the spotted ODT droplet has spread to form a larger spot. Here, the amount of thiol was insufficient to form a complete SAM. A good monolayer was formed only at the boundary line and in the center of the droplet.

the supply of thiols that a stamp can transfer to a Au surface. Compared to inkjet printing and μCP, the adsorption from solution is slower because it is more limited by diffusion.[4]

However, we found that the concentration of 1 mM was close to the lower limit to achieve a homogeneous monolayer coverage for this spot size. For more diluted solutions or when the droplets spread to form larger spots, the comet-tail effect disappeared, but parts of the spots were attacked by the etch; see Figure 3. Here, good protection by a SAM was obtained only at a 10 μm wide boundary line and in the center of the droplet. With slightly modified concentrations, one can achieve ring structures of different line widths and also without a central spot. Such patterns reflect the effects of transport within the droplets during their short drying phase.[6] When in addition the surface wettability switches from hydrophilic to hydrophobic during SAM formation, this can cause autophobic pinning or reactive spreading of the droplets.[25]

3.2. DNA Oligomers. The adsorption of thiolated oligonucleotides on Au is generally less ordered than those of alkanethiolate SAMs. Besides specific adsorption via the thiol−Au bond, these macromolecules may also attach unspecifically via nitrogen interactions of nucleotides[16] where the unspecific adsorption was found to be reversible during atomic force microscopy experiments in liquid.[20] In general, a molecular film consisting of disordered and partially loosely attached molecules potentially exposes numerous voids and would not be considered an efficient resist withstanding wet etch chemistry. Nevertheless, some wet etch recipes have been specifically optimized for use with molecular resist layers and ideally tolerate a low level of defects.[21] One of these selective etch methods, which is actually the same as shown above for the alkanethiols, also worked for DNA monolayers.

Droplets of aqueous buffer solutions containing 40 μM thiolated ssDNA oligomers were deposited by inkjet on gold surfaces. At relative humidities of 20−30%, the droplets deposited by inkjet evaporated from the substrates within a few seconds. The samples were rinsed with water to remove salt and excess DNA and then dried in a stream of nitrogen. Again, an ODT pattern was microcontact printed as a reference. Subsequent etching showed that the DNA deposit had excellent protective quality without any defects, thus proving homogeneous and dense coverage, Figure 4. Control experiments showed that TEAA buffer alone had no protective effect on the Au. Equivalent results were achieved with 12-mer, 20-

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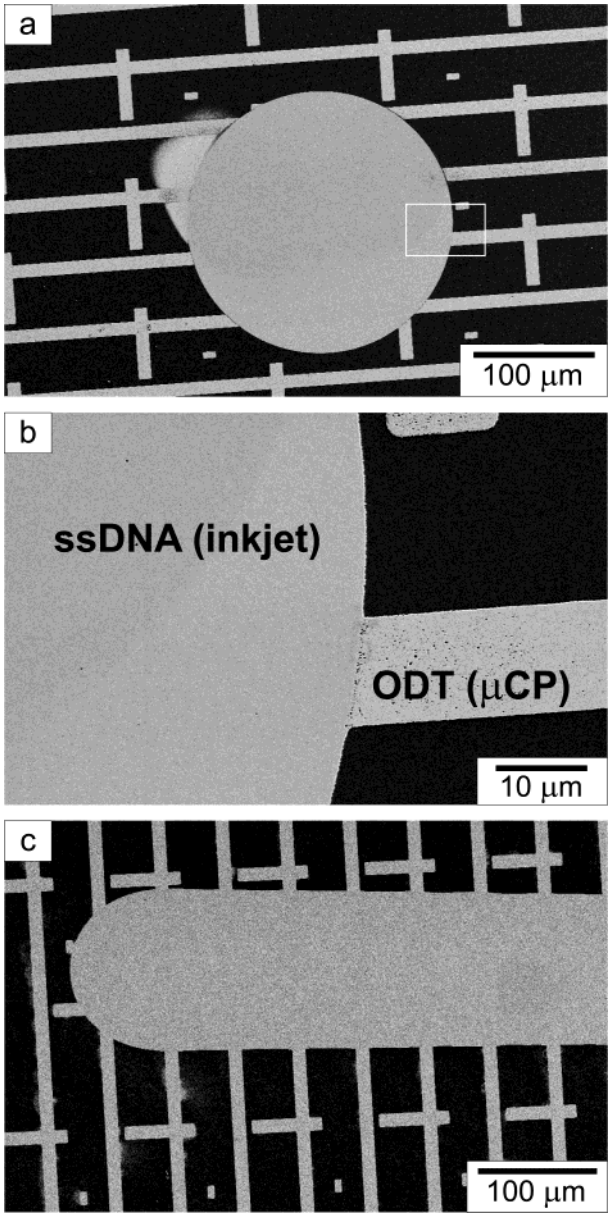

Figure 4. SEM micrographs of gold patterns generated by inkjet deposition of thiolated ssDNA 20-mer solution and μCP of ODT followed by a selective etch: (a) and (b) the ssDNA spots protected the gold perfectly without any defects; (c) a continuous line was achieved by spotting a sequence of droplets with a 50 μm pitch.

mer, and 30-mer ssDNA compounds, dissolved either in TEAA buffer or in DI water.

In principle, one can print arbitrary patterns with an inkjet. Figure 4c shows a straight line that has been spotted with excellent homogeneous coverage. Here, a series of droplets were deposited with a pitch of 0.05 mm. However, the wettability of the surface and the surface tension of the droplets can compromise the definition of arbitrary patterns. For example, the merging of droplets can create bulges on lines or at edges of the patterns, which is a general problem of printing on solid substrates.[26,27]

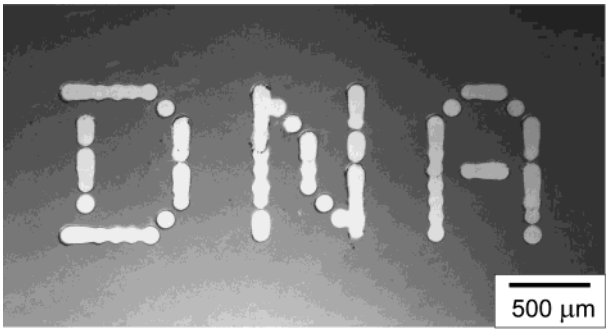

Figure 5. Optical micrograph of gold letters achieved by inkjet printing of non-thiolated ssDNA and subsequent etching.

As mentioned above, parts of the DNA strands are nonspecifically linked to the Au surface. If the thiol link were omitted, would the unspecific attachment be sufficient to resist being dissolved and provide a dense protective layer in the etch bath? In fact, when we carried out the same experiments with non-thiolated ssDNA oligomers, we found that the unspecific adsorption of ssDNA was strong enough to provide a similar degree of protection in the etch bath. Droplets of 20 μM solution of 30-mer ssDNA in water were used to print the characters "DNA". In the etch bath, the letters developed with excellent contrast, as can be seen in Figure 5.

4. Conclusion and Outlook

This study demonstrates that an inkjet is an efficient tool to create local SAMs of alkanethiolates or ssDNA monolayers on Au. The formation of homogeneous monolayer films by inkjet is remarkably fast. It takes only seconds compared to the several minutes recommended for immersion processes with similar concentrations. We introduced a wet-etch method to visualize the quality of these films. The wet etch was selective with respect to not only alkanethiolate SAMs but also thiol-functionalized and non-thiolated ssDNA monolayers. The residual Au structures reflected the integrity of deposited monolayer patterns and allowed microscopic inspection down to the nanometer scale. Our study also confirms that molecular resists can be deposited locally by inkjet in a direct-write scheme. This concept may be applied for the lithography of Au, Cu, and other metal layers. The functionality of the deposited layers will be addressed in further investigations. Our results suggest that inkjet technology is very useful for fabricating DNA microarrays and for functionalizing sensors such as cantilever sensors in a fast, reliable and reproducible way.[28]

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