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Replication of DNA submicron patterns by combining nanoimprint lithography and contact printing

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ABSTRACT

We describe a high throughput method of printing DNA submicron patterns on solid substrates. Combining nanoimprint lithography and contact printing, uniform DNA patterns can be produced with feature sizes from 2 μm down to 250 nm. In this method, poly(methylmethacrylate) (PMMA) with submicron line or dot patterns (produced by nanoimprint lithography) is first chemically functionalized with poly(ethyleneimine) (PEI) to aminate the surface and then a DNA pattern is prepared on the surface. PEI modification leads to higher DNA immobilization and DNA hybridization efficiency due to a higher density of amine functional groups provided by PEI. Next, complementary target DNA hybridized to the PMMA surface can be transferred to an aminated glass slide through contact printing. The transfer mechanism is due to electrostatic attraction (between DNA and amine groups) which can overcome hydrogen bonds between two hybridized DNA molecules. DNA transferred from the PMMA to the glass slide still can hybridize with DNA having a complementary sequence. This method provides a facile and high throughput means of preparing uniform DNA submicron patterns which are difficult to prepare by using conventional solution-based methods.

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1. Introduction

DNA arrays have rapidly become a useful tool for high-throughput genetic analysis in recent years [1–4]. To prepare a high-density DNA array, it is often necessary to dispense small droplets (∼nL) of buffer solutions containing synthetic DNA onto array surfaces, which allows the immobilization of DNA through the formation of chemical bonds between DNA and surfaces. However, during this process, it is difficult to control the evaporation of tiny droplets from surfaces. In some extreme cases, the evaporation of buffer from the surface may lead to the formation of doughnut-shape spots. To address the problem encountered in the solution-based procedure, a number of methods based on the contact printing of DNA by using polymeric stamps have been proposed by several groups [5–7]. For example, Lange et al. have shown that single-strand DNA molecules can be printed from a polydimethylsiloxane (PDMS) stamp to a solid surface as a new way of preparing DNA microarray. Compared to solution-based method, this new contact printing method is very fast and can be used to avoid irregular spots on the microarray. However, in this method, DNA solutions still need to be dispensed on the surface of PDMS stamps before printing, which poses a limitation on this method. Recently, a new method called Supramolecular nanoStamping (SuNS) has been developed independently by Crook's and Stellacci's groups to print DNA arrays [8–12]. In SuNS, the stamp surface is covered with an array of immobilized DNA probes that is complementary to the DNA targets need to be transferred to solid surfaces. After DNA targets hybridize on the stamp surface, they can be transferred to a solid surface through the formation of new chemical bonds between the DNA targets and the surface. Then, the two substrates can be separated by mechanical force or heating and DNA targets are left on the second substrate. The advantage of SuNS is that it does not require the dispensing of small droplets of DNA solution on the stamp surface. DNA targets can be captured onto the stamp surface at destinated locations through DNA hybridization. In addition, the replication of DNA array from a master surface can be achieved in a parallel way.

A common element in the techniques mentioned above is that it requires the chemical labeling of the DNA molecules with reactive functional groups such as amines or thiols. The functional groups are necessary because it allows the formation of new chemical bonds between the DNA molecules and surfaces. In contrast, our earlier study has demonstrated that the transfer of DNA from PDMS stamps to aminated glass slides can be achieved simply by using electrostatic attraction between negatively charged DNA and a positively charged surface [13]. The strong electrostatic interaction is able to overcome the hydrogen bonds between two strands

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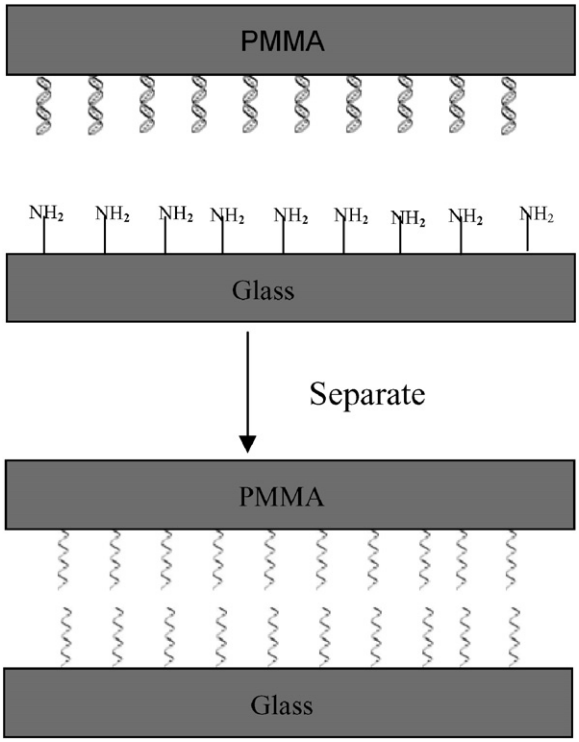

Fig. 1. Schematic illustration of the transfer of hybridized DNA from a PEI-modified PMMA surface to an aminated glass substrate.

of DNA and allow the transferring of DNA from the stamp surface to the positively charged surface during contact printing. However, PDMS is known to deform easily under a pressure. Therefore, it is difficult to use this method to prepare DNA array with features in the submicron range. In addition, to prepare a PDMS stamp suitable for printing of DNA is very time-consuming. After curing processes, the PDMS stamp needs to be cleaned thoroughly in ethanol for 24 h to extract all unreacted monomers from the stamp to avoid contamination problems [14]. In view of the disadvantages of PDMS, we consider PMMA as a better stamp material for printing DNA because PMMA is more rigid than PDMS. Despite of its mechanical strength, PMMA has a low glass transition temperature around 105 °C. The low glass transition temperature permits the preparation of nanostructures on the surface of PMMA with nanoimprint lithography, which has been applied to produce uniform nanoscale patterns on surfaces in the past [15,16]. However, to the best of our knowledge, PMMA only had been used as a substrate for DNA microarray in previous studies [17–19] (e.g., Yu et al. printed DNA microarrays on a PMMA substrate by using the SuNS technique [11]). It has not been used as a stamp for printing DNA before. In this paper, we will first study the feasibility of forming a high-density DNA pattern on the surface of PMMA. Next, we will employ nanoimprint lithography to prepare PMMA stamp with submicrometer features on the surface. Finally, we will hybridize DNA on the PMMA surface and use the PMMA-based stamp to print submicrometer-size DNA patterns by using contact printing method as shown in Fig. 1.

2. Materials and methods

2.1. Materials

Commercial PMMA sheets (thickness 0.5 mm) were obtained from Goodfellow (UK) and all glass slides were purchased from Marienfeld (Germany). The PMMA sheets were cut into pieces with desired dimensions (2 cm × 2 cm), then rinsed with 2-propanol to remove surface contaminants. PEI ($M_w = 75{,}000$), hexamethylenediamine (HMD), (*N*-(3-(trimethoxysilyl)propyl)-ethylenediamine) (AEAPS), borate buffer were purchased from Sigma-Aldrich (Singapore). Methanol (HPLC grade) was purchased from Merck (Germany). All modified DNA was purchased from Research Biolabs (Singapore). Their detailed sequences were shown in Table 1. DNA sequences (**D1** to **D7**) are biomarker for cya and lef genes from *B. anthracis* [20]. All aqueous solutions were prepared in deionized water with a resistance of 18.2 MΩ.

Table 1
DNA probes and targets used in this study.

Symbol	Sequence
D1	NH_2-5′-CTGCATGTTCTGGTACTAAACCTGA-3′-FAM
D2	NH_2-5′-CTGCATGTTCTGGTACTAAACCTGA-3′
D3	FAM-5′-TCAGGTTTAGTACCAGAACATGCAG-3′
D4	NH_2-5′-GTGGCTCGATATAATATGCAAAAGC-3′
D5	FAM-5′-GCTTTTGCATATTATATCGAGCCAC-3′
D6	5′-TCAGGTTTAGTACCAGAACATGCAG-3′
D7	FAM-5′-CTGCATGTTCTGGTACTAAACCTGA-3′

2.2. Nanoimprinting process on PMMA

Imprinting was performed by using a nanoimprinter (Obducat, Sweden) to create patterns on PMMA sheet. Before use, a Si mold with desired patterns was cleaned with acetone, isopropanol and oxygen plasma (80 W, 250 mTorr for 2 min). The mold was then pressed against PMMA sheets at 110 °C, 20 bar for 300 s.

2.3. Chemical modifications of PMMA and glass slides

To modify the PMMA surface with PEI for DNA immobilization, pristine PMMA sheets were immersed in 100 mM borate buffer (pH 11.5) containing 1 wt% PEI at room temperature for 2 h [21]. After the reaction, they were immersed in DI water for 30 min, sonicated, and dried with nitrogen. In the second procedure, PMMA substrates were modified with HMD in 100 mM borate buffer containing 10% (w/v) HMD for 2 h [15], followed by rinsing with DI water for 15 min and dry with nitrogen as shown in Figs. 2a and 2b. Both aminated surfaces were then activated in 5% (w/w) glutaraldehyde solution containing 10 mM sodium cyanoborohydride (as a reducing agent) at room temperature for 2 h. Finally, the aldehyde-terminated PMMA surfaces were washed with DI water and dried with nitrogen. To introduce amino groups to the glass surface, we used AEAPS for surface modification. The glass slides were immersed in an aqueous solution containing 5% (v/v) of AEAPS at 50 °C for 2 h. After washing with DI water for 15 min, glass slides were baked in a vacuum oven at 100 °C for 15 min to promote the crosslinking of silanol groups.

2.4. Immobilization and hybridization of DNA on PMMA surfaces

Amine-labeled DNA probes in 0.5 M carbonate buffer containing 10 mM sodium cyanoborohydride was dispensed onto the aldehyde-terminated PMMA surface. The PMMA substrates were incubated in a humid chamber at 50 °C for 2 h to promote the linkage between the amine group of DNA and aldehyde group on the PMMA surface. After the incubation, the PMMA surface was rinsed with 2 × SSC (containing 0.02% SDS) and methanol sequentially. Finally, the surface was washed with DI water and dried under a steam of nitrogen gas. To hybridize DNA, the PMMA substrates decorated with DNA probes were first immersed in 0.5 M carbonate buffer containing 0.1 M succinic anhydride for 2 h to block unreacted surface amine groups. Then the samples were incubated in TE hybridization buffer (1 M NaCl, 10 mM Tris-HCl, 1 mM EDTA,

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