

Polyurethane Molecular Stamps for the *in situ* Synthesis of DNA Microarray

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Abstract: Fabrication of polyurethane molecular stamps (PU stamps) based on polypropylene glycol (PPG) and toluene diisocyanate (TDI), using 3, 3'-dichloro-4, 4'-methylenedianiline (MOCA) as the crosslinker, is reported. It was shown from the contact angle measurement that PU stamps surface has good affinity with acetonitrile, guaranteeing the well distribution of DNA monomers on patterned stamps. Laser confocal fluorescence microscopy images of oligonucleotide arrays after hybridization confirmed polyurethane is an excellent material for molecular stamps when transferring polar chemicals and conducting reactions on interfaces by stamping.

Keywords: Molecular stamps, polyurethane, contact angle, soft lithography, DNA microarray.

After the completion of the sequence of the human genome¹, a greater challenge than gene sequencing is to uncover sequence information, to relate these messages to the mechanisms of the various biological processes². To achieve these goals, an efficient approach is to assemble oligonucleotide arrays (ONAs) containing a variety of addressable sequences for high throughput applications in genetic, biomedical, and biochemical areas. An increasing number of methods for the preparation of ONAs have been reported²⁻¹². The most successful approach among these methods was pioneered by Fodor and his coworkers¹¹, which is based on photolithography, a technique borrowed from the semiconductor industry. The critical issue is that the dimethoxy trityl (DMT) group which protects the 5' hydroxyl is replaced by a photolabile protecting group. The gene chips fabricated with this method are commercially available now. However, to develop new cheap and more efficient methods for *in situ* synthesizing DNA microarray is still in need.

Our group developed a stamping method for the *in situ* synthesis of DNA microarray¹³. DNA microarray synthesis using molecular stamps is in fact a kind of quasi-solid phase interface reaction with contact printing and combinatorial chemistry experiment. The solutions of monomers are spun onto the patterned surface of stamps

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and dried under inert gas atmosphere. Then the patterned surface was pressed onto the designed substrate such as glass slides modified with linkers, and the monomers remained on stamps are allowed to react with the linkers. Well-distribution of reaction solution on the surface of stamps is a critical factor to keep the homogeneity of the reaction on the substrate, which requires proper material-made stamps. Good stamps can also guarantee the accurate overlap of the patterns of a set of stamps with which the DNA microarrays were synthesized *in situ* on glass slides. PDMS (polydimethylsiloxane) is an excellent material used in soft lithography¹⁴. However, the monomers are usually dissolved in polar solvent (*e.g.* acetonitrile), the non-polar PDMS stamps can not be used directly to fabricate DNA microarray due to the hydrophobicity of the PDMS stamps. Although microwave plasma can induce a hydrophilic surface¹⁵, but its polarity can only remain a few days even after grafting,. In order to overcome the difficulties and limitation upon a PDMS stamp, we have developed a novel stamp from polyurethane to fabricate DNA microarray *in situ*.

Typical experimental procedure

The polypropylene glycol (100 g 0.05 mol) was dried under vacuum at 120°C for one hour. The catalyst DBTDL 0.3 g was added to the polyol and PPG2000 was allowed to react with a calculated quantity of the diisocyanate TDI(33 g 0.075 mol) at 40°C in five neck reaction kettle fitted with a mechanical stirrer and nitrogen inlet. The diisocyanate was added dropwise from the dropping funnel over a period of 20 min. The mixture was immersed in an oil bath at 80°C and stirred continuously for 2 h to obtain NCO terminated prepolymer.

The master prepared by using conventional photolithography was employed as the motherboard with 10000 rectangles per square centimeter and the thickness of 3.5×10^{-5} m. The prepolymer (100 g) was mixed with melt MOCA (12.2 g) at 80°C, then the mixture was poured onto the prepared motherboard, then degassed quickly and strictly (vacuum degassing is critical to the success of the process). Then a piece of glass was put on it and the curing was allowed to proceed at room temperature for 48 h. After cooling the crosslinked polyurethane with the cover was carefully peeled from the motherboard and the PU stamps came into being.

Results and Discussion

Goniometric analysis provides a relatively quick and simple means of assessing the affinity of PU stamps with liquids. Equilibrium contact angle of water and acetonitrile with PU stamps were measured using a Rame-Hart 100R contact angle instrument. *Prior to* measurement the PU stamps were rinsed with double distilled water and dried under a stream of N₂. Measurements were made on sessile drops (1μL droplets) by measuring the tangent of the droplet to the PU stamps surface at intersection position. Ten PU stamps were used to evaluate their affinity to water & acetonitrile (5 respectively). Each reported value is the mean contact angle for ten sites of every stamp. Measurements of contact angle were taken within 10 s after formation of the sessile drop. The results were listed in **Table 1**. It was shown that although owing to the relative high water contact angle (63.8°), which gives dry PU stamps easily in aqueous atmosphere, the prepared PU stamps

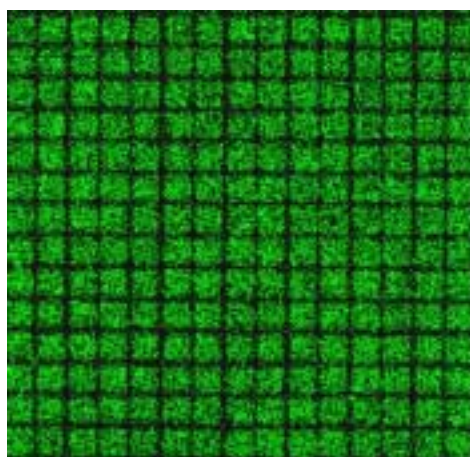
have good affinity to acetonitrile (acetonitrile contact angle is 2.4°), which guarantees the well-distribution of DNA monomers solution on the surface of stamps and then keeps the homogeneity of the reaction on the substrate.

Table 1 Water and acetonitrile contact angle of PU stamps ($^\circ$)

Sample	1	2	3	4	5	Even
Double distilled water	63.5	64.2	62.5	65.6	63.5	63.8
Acetonitrile	2.5	2.2	2.4	2.5	2.2	2.4

DNA microarray with the sequence of 3'-TTTATCAGTACGACTATGTC-HEX was synthesized with the method of molecular stamping mentioned in the document¹³ by utilizing the prepared PU stamps reported here. Then the DNA microarray was hybridized with the relevant complementary DNA strands and was examined using laser confocal fluorescence microscope. A part of the obtained high definition fluorescence microscopy image is shown in **Figure 1**. From this image, we can find every rectangle presents a strong bright fluorescent signal with a high contrast to the background, meaning the chemicals reacted well on the interface upon stamping. This satisfactory result should be attributed to the well distribution of chemicals on the polar surface of the PU molecular stamps. Therefore, PU stamps reported here perhaps are the ideal tools for *in situ* synthesizing DNA microarrays by stamping.

Figure 1 Laser confocal fluorescence microscopy images (part) of oligonucleotide arrays (3'-TTTATCAGTACGACTATGTC-HEX) synthesized by using PU stamps after hybridizing with its complementary DNA strands.



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