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# Dynamic PDMS inking for DNA patterning by soft lithography

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ABSTRACT

Microcontact printing (μCP) is used as a patterning technique to produce simple, rapid and cost-effective DNA microarrays. The accuracy of the final transferred pattern drastically depends on the inking step. The usual way to ink a PDMS stamp by droplet deposition of labeled biomolecules using a pipette, results in irregular transfer of the biomolecules on the chip surface and leads to poor and irreproducible fluorescent signals. These drawbacks are likely due to irregular 'coating' of the biomolecules on the PDMS stamp. In this work, a novel approach for inking PDMS with DNA molecules is presented. It is based on the continuous displacement of the meniscus formed by the inking solution over the surface of the stamp. When compared with the conventional technique, this dynamic PDMS inking method proved to be very reproducible for producing regular prints/spots on a functionalized glass slide, and this method could be easily extrapolated at an industrial scale.



## 1. Introduction

Conventional techniques of DNA microarrays fabrication such as spotting needles [1], ink jet nozzles [2,3] or direct *in situ* synthesis of oligonucleotides on glass slides [4] require sophisticated equipment, are time consuming and relatively expensive. Microcontact printing (μCP) technique using an elastomeric stamp, made preferentially in PolyDiMethylSiloxane (PDMS, Sylgard 184) has been proposed as an alternative and cost-effective procedure of fabrication, easy to implement and potentially transposable at an industrial scale [5,6]. In addition, the μCP technique was found to be useful for patterning of any kind of biomolecules including proteins, bilipidic membranes or even single cells onto functionalized surfaces [7–9]. However, a major critical step for successful application of this technique for biochips fabrication at industrial scale is the inking of PDMS stamp with DNA molecules allowing a perfect, regular and reproducible transfer of the biomolecules on the surface for optimal hybridization and henceforth for reproducible and robust biochips. The usual way for inking PDMS stamps is based on the deposition of a drop containing the DNA molecules over the patterned surface of the stamp using a micropipette. This inking system leads to irregular 'coating' of the stamp likely due to several reasons, among which, the opposite chemical nature of PDMS which is hydrophobic while DNA is mostly hydrophilic and the uncontrolled drying of the deposited drop. This lack of regularity in the PDMS 'coating' with DNA molecules leads to poor hybridization signal and constitutes a real drawback for a robust and reproducible biochip fabrication, preventing its industrial development.

To overcome this problem, we present in this paper a new approach for inking PDMS stamp that is based on a continuous displacement of the biomolecules solution meniscus over the surface of the stamp. This automated inking system allows tight control over the displacement of the front and the rear menisci at a defined speed and meniscus angle. This novel inking technique was compared with the classical mode of inking using fluorescent DNA probes. Optical microscopy revealed a remarkable reproducibility and regularity of the micrometric spots made with our novel PDMS inking technique, as characterized by a weak variation in the fluorescence intensity (CVs) between the spots.

## 2. Material and methods

### 2.1. Materials

DNA probes were 24-mer oligonucleotides modified with a 5′-amino $C_6$ linker for covalent linkage to the dendrislide [10]

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and labeled with a 3′-Cy3: (5′[AmC$_6$T]-ATACTCCGGGAAACTGA-CATCTAG-[Cy3] 3′) (Sigma–Aldrich) to allow detection using a fluorescence reading scanner (InnoScan® 710, Innopsys). The DNA probes were HPLC purified (Sigma–Aldrich) and diluted at final concentration of 1 μM in a sodium phosphate buffer (PBS) at pH 9.

### 2.2. Fabrication of master mold and stamps

The fabrication of a PDMS stamp requires the generation of a silicon master mold (Fig. 1). The micrometer-patterned master mold was achieved by UV photolithography and the pattern transfer by reactive ion etching (RIE) where the targeted etch depth was fixed to 160 μm. An anti-adhesive treatment is carried out using silanisation in liquid phase with OTS (OctadecylTrichloroSilane) to facilitate the master demolding. The PDMS prepolymer solution composed of a mixture of PDMS oligomers and a reticular agent from Sylgard 184 Kit (Dow Corning) (at mass ratio 10:1) was poured on the silicon master and cured at 80 °C for 6 h. The micrometric patterns used are blocks of 25 pillars (5 × 5, 160 μm diameter with a pitch of 300 μm) [6].

### 2.3. Inking step

#### 2.3.1. By droplet deposition

DNA probes solution (200 μl at 1 μM in sodium phosphate buffer) was deposited on the surface of the microstructured PDMS stamp during 1 min using a micropipette, followed by removal of the excess of DNA solution with the micropipette. Then, the stamp was blown dry under a stream of nitrogen.

#### 2.3.2. By dynamic PDMS inking

The microstructured PDMS stamp was placed on a motorized translation stage. A DNA reservoir for the inking solution was formed by two hydrophobic plastic cover slips (Deckgläser, Knittel-Glass, 24 mm × 32 mm) separated by 1 mm plastic wedges that were glued together (using Super glue 3 Loctite) onto the cover slips. A 20 μl DNA solution at 1 μM was injected between the two hydrophobic plastic cover slips using a micropipette. The DNA reservoir was positioned in contact with the surface of the PDMS stamp at a defined angle of 15°, while the stamp was placed on a computer control motorized platform (NewPort MFA-CC). This system allowed mechanical displacement at a given constant velocity ranging from 0 to 2.5 mm/s by step of 1 μm/s. The maximum displacement range was 25 mm. Dynamic PDMS inking was performed throughout the entire surface of the PDMS stamp (8 mm × 14 mm). The experiment was conducted at room temperature. The meniscus contact angles during dynamic inking were measured using Digidrop from GBX. All experiments were performed at ambient temperature (22 °C) and at 52 ± 2% of humidity.

### 2.4. Transfer of DNA molecules on biochips

The stamp was brought into contact with the dendrimer-coated glass slides (dendrislides) [10] for 1 min without external pressure and then peeled away. The microstructured PDMS stamp leads to a pattern of DNA molecules composed of 6 blocks of 25 spots. The spots were 160 μm diameter and formed a 300 μm pitch grating of 25 × 25 spots.

### 2.5. Optical microscopy

Bright-field images were acquired with a Nikon eclipse LV 100 microscope, equipped with long-distances bright-field lenses and coupled with an Andor Luca EMCCD camera (Andor™ Technology); and images were captured using Andor Solis acquisition software.

### 2.6. Fluorescence images acquisition

The fluorescence images were obtained using an InnoScan®710 fluorescence reading scanner from Innopsys SA (Carbonne, France). This scanner provides uniform scanning across the whole microarray surface using confocal PMT detection (laser excitation wavelength at 532 nm or/and 635 nm) and a real time autofocus. The scanning parameters were PMT 532 at 5% and PMT 635 at 100% and brightness/contrast/balance at 50/10/50 respectively. The fluorescence intensity of each spot of a block was acquired using the Mapix software. The homogeneity of the printed DNA was estimated by determining the fluorescence intensity variation coefficient (CV), which is the standard deviation between the 25 spots

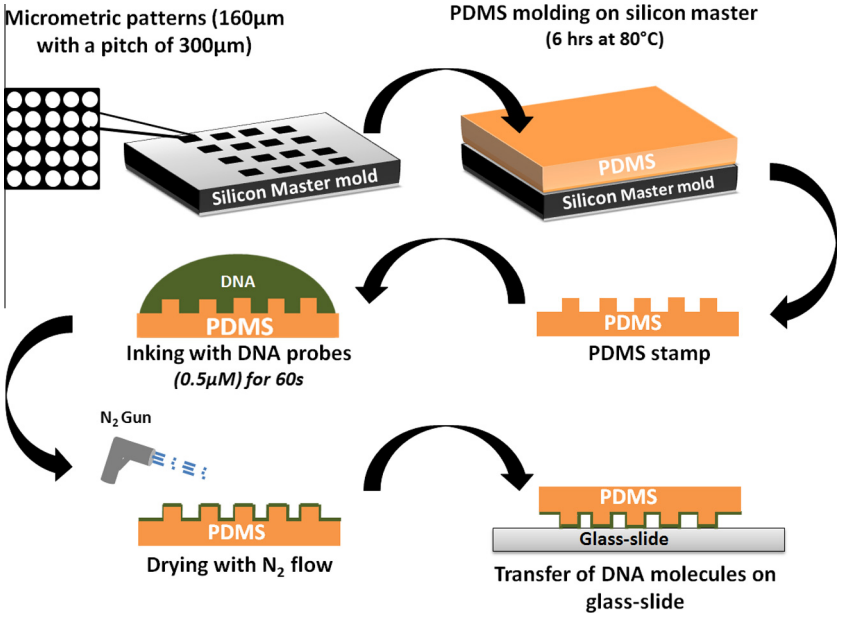


**Fig. 1.** Scheme of the microcontact printing process.