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Benchtop Nanoscale Patterning using Soft Lithography for the Printing of DNA Molecules

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***Abstract*— DNA microarrays have rapidly evolved to become one of the essential tools to examine Expression or mutation of thousands of genes simultaneously. The microcontact printing (μCP) could fulfill this requirement as it is a printing technology that uses inexpensive Elastomeric stamps made usually of polydimethylsiloxane (PDMS) and which exhibits relief patterns at the micron and Nano scale. In this work we made a benchtop nanoscale pattering using soft lithographic techniques and made a PDMS stamp. This stamp is characterized using Atomic force microscope and Laser diffraction technique to confirm the nanoscale patterns. Genomic and plasmid DNA (Maxprep) are isolated from Ecoli cells and these biomolecules are immobilized on solid surfaces such as surface modified microscope glass slide and silicon wafers by micro contact printing. These printed surfaces are characterized and analyzed by Atomic Force Microscopy (AFM). The surface bound biomolecules find application in bio sensors, chromatography, diagnostic immune assays, cell culturing, expression studies and other analytical procedures.**
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Keywords*— *Poly dimethyl siloxane (PDMS), Microcontact printing, DNA molecule, Atomic force microscopy, Soft lithography.

I. INTRODUCTION

Soft lithography refers to a family of techniques for fabricating or replicating structures using elastomeric stamps/ molds. It is called "soft" because it uses elastomeric materials with increasing interest in nanoscale science and technology. The development of new and inexpensive fabrication techniques to generate nanostructures has become an important scientific challenge. We have demonstrated a simple benchtop nano patterning technique to print DNA molecules into different solid substrates [1].

The key element in Micro contact printing (μCP) is a polymeric stamp, i.e. a slab of polymer that bears a microscale relief pattern on one side [2]. This stamp is ‘‘inked’’ and put in contact with the substrate surface. Ideally, the ink is transferred from stamp to substrate only in the area of contact. Poly (dimethylsiloxane) (PDMS) is the most widely used material to make μCP stamps. PDMS, as an attractive polymeric material, was first introduced by Effenhauser et al for the separation of DNA fragments in 1997. Since then, it has become a popular material for building micro-fluidic devices mainly due to its excellent optical transparency, easy replica molding, nontoxicity, low electrical conductivity, low cost, increasing versatility, and biocompatibility. It also has the property of sealing either reversibly or irreversibly to many materials at room temperature.[3] However, there are also several disadvantages to the use of PDMS. Micro-fluidic devices such as easy generation of air bubbles in channels, unstable and extreme hydrophobicity of PDMS that makes the surface difficult to wet with aqueous solution. Furthermore, due to its high hydrophobicity, PDMS adsorbs some organic solvents and some hydrophobic analytes, causing fouling of the material. These disadvantages greatly limit the variety of analyses that can be separated on PDMS devices, and generally result in relatively lower separation efficiency. PDMS has a number of properties which are very well suited for μCP. PDMS is flexible enough to make conformal contact even with rough surfaces but still shows enough mechanical stiffness to reproduce patterns in the micro metre range. The Young’s modulus of a PDMS stamp is typically around 1.5 MPa. Moreover, PDMS stamps can be produced rather easily by thermally curing the pre-polymer for a few hours. Finally, PDMS is commercially available (Sylgard-184) [4]. While printing more polar molecules, especially biological ‘‘inks’ ‘such as proteins or DNA, the ink is repelled by the hydrophobic PDMS stamp, which means that the stamp is not sufficiently inked and as a consequence the ink cannot be transferred to the substrate. This problem can be faced either by surface treatment of the PDMS stamps (e.g. oxidation) or chemical modification.

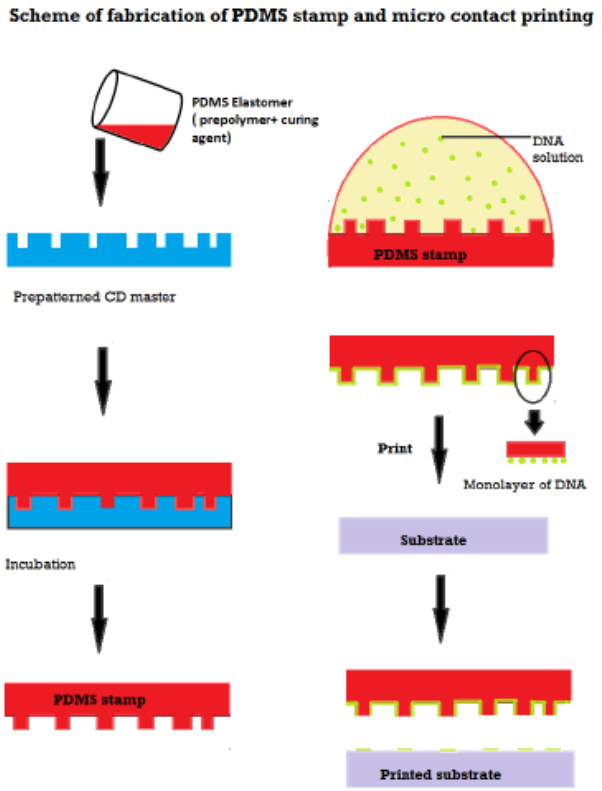


Figure I: Schematic representation of PDMS stamp fabrication and Micro contact printing

II. EXPERIMENTAL

A. Preparation of Nanoscale masters

The first exercise in soft lithography is to fabricate a master. Masters are high-quality patterns that are typically fabricated using time-consuming and expensive techniques such as photolithography, e-beam lithography, and focused ion beam milling. We have found a simple and economic alternative for obtaining masters with nano scale features of CDs. CDs are composed of multiple layers and two adjacent layers can be used as two masters [5], in which one layer has features inverted to that of the other. It is shown in figure I.

B. Isolation of DNA from bacteria

When scientists study DNA, cells are chemically lysed (broken open) and DNA from chromosomes is released. This procedure is known as cell lysis. The DNA is then isolated from a solution and re-dissolved. E. coli chromosomal DNA is a large circular molecule containing approximately, 3000,000 base pairs attached to the bacterial cell wall at several points [6]. Large DNA is very sensitive to mechanical shear which causes random breaks in the phosphodiester backbone of the molecule. However, if the extraction procedure is performed carefully, large fragments of chromosomal DNA can be obtained with an average fragment length of 100,000 to 200,000 base pairs. Since the average length of a gene is about 2,000 base pairs, there is a high probability that genes of interest will remain intact in one of the fragments of DNA.

Plasmids are small double-stranded pieces of DNA that exist outside the nucleus of the cell in which they are located. Almost all plasmids are circular. They contain a small number of genes, which can include genes that make cells resistant to antibiotics. Plasmids are used extensively in cloning of DNA. There are several ways to purify plasmids. These methods are often called “Maxipreps” because we are purifying DNA from a large volume of cells. Maxipreps involve lysing the cells and purifying the DNA via centrifugation and/or membrane binding. First purify the plasmid DNA and analyze it by using agarose gel electrophoresis. Agarose gel electrophoresis is a convenient procedure for analyzing DNA. DNA has a strong negative charge at neutral pH; it will migrate through the gel towards the positive electrode. Pores in the gel separate linear fragments of DNA according to their size. Then DNA sample is fragmented around 100-1500 bp size for stamping process. One method for DNA fragmentation is by sonication. During sonication, DNA samples are subjected to hydrodynamic shearing by exposure to brief periods of sonication. These fragmented DNA solution is our sample to print on a substrate.[7]

Micro contact printing

Printing (µCP) Sophisticated version of a simple stamping process that is familiar even to most children. Similar to conventional printing, µCP also involves an ink, a substrate and a stamp. In contrast to the dyes that are normally used for printing, inks for µCP are DNA printed in monomolecular layers. Instead of paper, clothing, or wood, the surfaces for µCPare usually ultra-flat metal, silicon or glass substrates. [8]

As an alternative to photolithography, Whiteside and coworkers introduced in 1993 a novel method called micro contact printing (µCP) for patterning self-assembled monolayers of alkanethiols onto gold substrates. Their technique shares some similarities with that used in the paper printing industry, where it is termed flexography [9]. The authors used polydimethylsiloxane (PDMS), an elastomer, to form a patterned stamp, which transfers ink made up of molecules such as alkanethiols locally to gold surfaces. The printed self-assembled monolayer (SAM) of thiols can serve to control the wetting properties of the gold surface, the nucleation of crystals, or the etch resistance of the underlying substrate. Using this approach, these authors structured silicon, for example. [10]. Hence, µCP and derived techniques are now referredto as soft lithography. µCP is a simple, flexible and efficient patterning technique for bio molecules. [11]. we are using DNA for efficient patterning.

Emerging microarray technology allows the expression of thousands of genes to be studied simultaneously. This has become possible by attaching DNA molecules to the surface of a microscope slide arranged in an array format. In order to obtain purified DNA from cells and to study it, the DNA must be separated from the rest of the cellular material. [12]

III. RESULT AND DISCUSSION

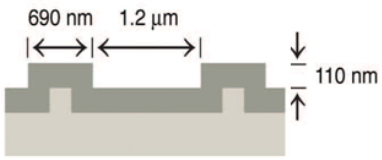
We have demonstrated a simple benchtop nano patterning technique to print DNA molecules into silicon substrate. The key element in Micro contact printing (µCP) is a PDMS stamp. PDMS has a number of properties which are very well suited for µCP. PDMS is flexible enough to make conformal contact even with rough surfaces but still shows enough mechanical stiffness to reproduce patterns in the micro metre range.

A. AFM image analysis of the PDMS stamp

AFM pictures were recorded using a Nanoscope Dimension 3000 (Digital Instruments, Santa Barbara, CA) operated in tapping mode using Super Sharp Silicon tips (Nanosensors, Norderfriedrichskoog, Germany) having a tip radius <5 nm (guaranteed by the manufacturer). All images for measuring the dimensions of patterns were taken with the same tip. The same pattern was recorded at the beginning and at the end of each series of experiments to verify that no change in resolution had occurred during the experiments.

The AFM images obtained is given in Figure II (A). The scan size is 8×8 µm. From AFM analysis we got the information that the stamp surface has bumps that are 690 nm wide, 110 nm high, and spaced by 1.2 µm. For the better understanding of the stamp surface, it is represented in Figure II (B)

(B)



(A)

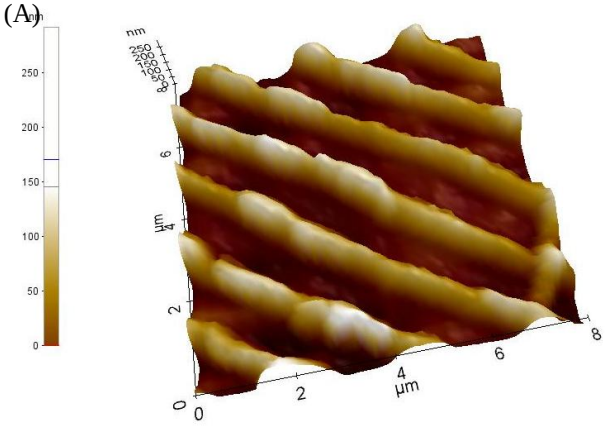


Figure II (A) AFM image of the PDMS stamp (B) Cartoonic representation of the stamp surface

B. Studies of Optical Diffraction of Laser in PDMS Stamp

Laser beam is passed through the PDMS stamp and the diffraction pattern is studied. The distance between any two of these outgoing waves from the successive grooves depends on the wavelength of the wave, as well as the density of grooves, or the distance between the previously mentioned slits or holes. Use the Fraunhofer equation,

$$2d \sin \varphi_n = n \lambda \quad (1)$$

To determine the feature spacing, *d*, of the original transparency and its copy by passing a laser with wavelength λ through the sample and measuring the *n*th diffraction angle,

$$\varphi_{n=X/L} \quad (2)$$

We got only the first order diffraction pattern. The obtained pattern consists of 3 laser spots in a row. It indicated that the stamp consist of linear grooves. The separation between the groves is found to be 1.2 µm using the equations (1) and (2)

C. Agarose Gel Electrophoresis studies

Genomic DNA and plasmid DNA are isolated by the *Maxipreps* methods. The gel was run and it is placed in the trans illuminator. Clear fluorescent bands of Genomic and plasmid DNA were observed. Thus the Genomic and Plasmid DNA was successfully isolated and detected. We confirmed the presence of DNA using agarose gel electrophoresis. The images obtained are shown in Figure III, and Figure IV respectively.

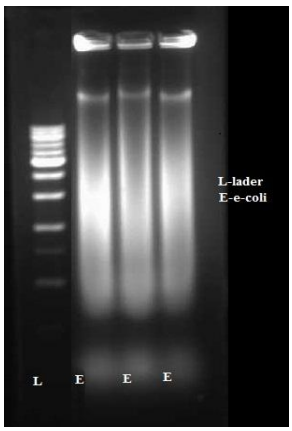


Figure III: Image of agarose gel electrophoresis of Genomic DNA

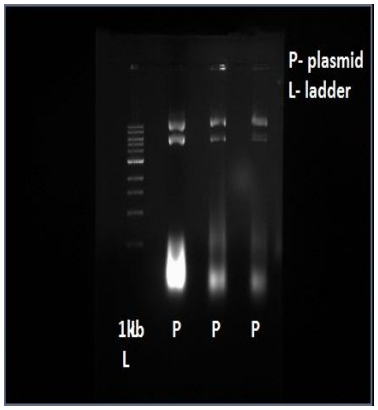


Figure IV: Image of agarose gel electrophoresis of Plasmid DNA

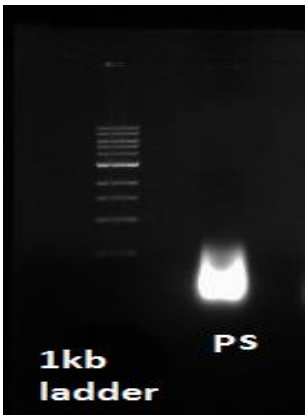


Figure V: Image of agarose gel electrophoresis of the sonicated Plasmid DNA

In biological applications, sonication is sufficient to disrupt or deactivate a biological material. Sonication is also used to fragment molecules of DNA, in which the DNA subjected to brief periods of sonication is sheared into smaller fragments. We sonicated the DNA sample. From the agarose gel electrophoresis studies it is observed a smear of plasmid DNA down the lane. It represents large pieces of plasmid DNA that are partially degraded (broken down) into thousands of smaller fragments or "cut" plasmid and the fragment length is nearly 500-1000 bp. The image is shown in Figure.V.

D. AFM image analysis of the printed DNA molecule

PDMS Stamp is modified using the PDMS surface modification kit from Sigma. This kit results in a durable hydrophilic PDMS surface. The resulting surface should be wettable by hydrophilic inks and appropriate for multiple printing applications. The genomic DNA is loaded on to the modified PDMS stamp and its AFM images are taken (see Figure VI) and compared with that of empty stamp in Figure II (A).The presence of DNA is clearly observed. The AFM image of the printed DNA on cleaned silicon wafer is analyzed. The image is shown in Figure VII. The printed silicon wafer were undergone AFM image analysis, the observed results are shown below.

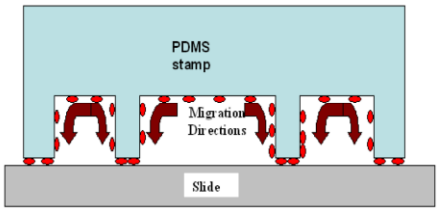


Figure VII: Migration of the DNA molecule to the contact surface

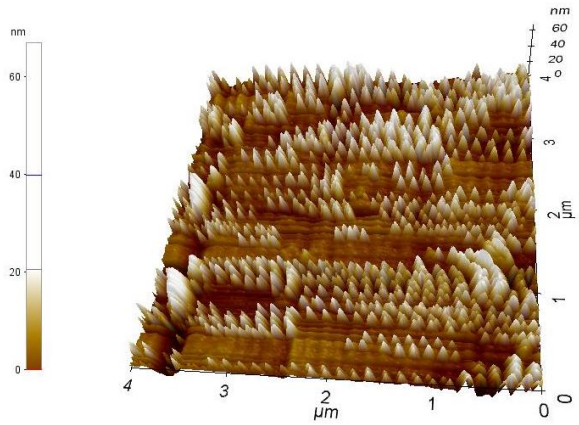


Figure VI: AFM image of the stamp loaded with Genomic DNA

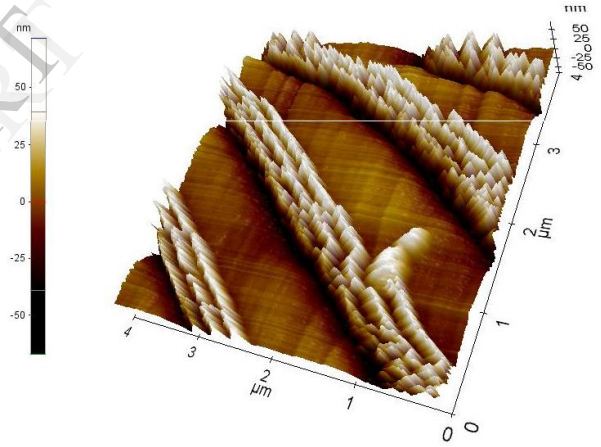


Figure VIII: AFM image of the printed silicon surface with Genomic DNA

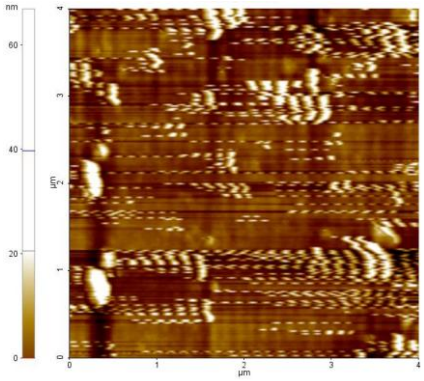


Figure IX: AFM image of the stamp loaded with Plasmid DNA

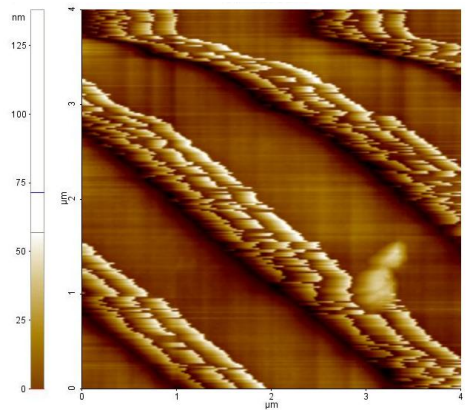


Figure 3.10: AFM image of the printed silicon surface with Plasmid DNA

The excellent performance of this technique to print DNA is the interaction takes place between the PDMS surface of the stamp and DNA molecules. Since the PDMS surface is highly hydrophobic, and the DNA strand can also exhibit hydrophobic properties we have to convert it into hydrophilic by surface modifications. Transfer of the DNA molecules from the stamp to the slide required that the interacting forces between the oligonucleotides and the PDMS surface must be weaker than those occurring between the oligonucleotides and the surface of the slide. This was verified in our experiments

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CONCLUSION

DNA microarrays have rapidly evolved to become one of the essential tools to examine Expression or mutation of thousands of genes simultaneously. There is a need for alternative Patterning methods that must be very simple, reproducible, cost-effective, and eventually Transferable to any laboratories for their own problematic situations. The microcontact printing (µCP) could fulfill this requirement as it is a printing technology that uses inexpensive Elastomeric stamps made usually of polydimethylsiloxane (PDMS) and which exhibits relief patterns at the micron and Nano scale. Microcontact printing has been demonstrated as a technique for the parallel delivery of proteins as surface patterns onto a target substrate. A stamp made of an elastomeric material such as poly (dimethylsiloxane) (PDMS) can be topographically structured by casting the prepolymer against a 3D master. The stamp is inked with the molecules of interest, forming a more or less complete monolayer, rinsed with buffer, blown dry under a stream of nitrogen, and then printed onto the substrate surface. The two main steps of µCP are the adsorption of the DNA on the stamp (inking process) and the transfer of ink from the stamp to a target surface (contact printing). The contact times were as short as possible for optimizing the high throughput of the technique. In this work we made a benchtop nanoscale pattering using soft lithographic techniques and made a PDMS stamp. This stamp is characterized using Atomic force microscope and Laser diffraction technique to confirm the nanoscale patterns. The bumps are 690 nm wide, 110 nm high and spaced by 1.2 µm. Genomic and plasmid DNA (Maxprep) are isolated from Ecoli cells using standard protocols and these biomolecules are immobilized on solid surfaces such as surface modified microscope glass slide and silicon wafers by micro contact printing. These printed surfaces are characterized and analyzed by Atomic Force Microscopy (AFM). The surface bound biomolecules find application in bio sensors, chromatography, diagnostic immune assays, cell culturing, expression studies and other analytical procedures.

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