Fabrication of Elastomeric Nanofluidic Devices for Manipulation of Long DNA Molecules

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Abstract. We propose a method for the separation of long DNA molecules, based on elastomeric nanochannels with tunable cross section. These nanoconfinement structures can be used to stretch DNA molecules and lower their conformational entropy. The sieving mechanism of entropic recoil, proposed by Cabodi et al. [1], will be implemented using an array of elastomeric nanocheannels. Structures of various dimensions are fabricated taking advantage of replica molding techniques, starting from Focused Ion Beam (FIB) patterned silicon substrates. Poly(dimethylsiloxane) (PDMS) and hard-PDMS [2] are used to replicate the features on the silicon mold. After plasma oxidation the nanochannels are sealed using a glass cover slip. A piezoelectric system will be integrated on the device in order to exploit the elastomeric propertis of PDMS, reversibly deform the nanochannels and tune their cross section. This system will allow a dynamic variation of the confinement conditions affecting molecules mobility inside the nanochannels.

Keywords: Nanochannels, PDMS, DNA Separation, Nanoconfinement.

1 Introduction

Nanoconfinement is widely used for separation of DNA molecules. When a DNA strand is forced to enter a confined space, it must lower its conformational entropy and it acquires a stretched configuration. In the past decade, variations of DNA molecule conformational free-energy have been widely exploited for sieving applications [1],[3]-[6]. In 2007, Huh et al. [7] proposed an interesting and low cost approach for the fabrication of PDMS nanostructures for nanofluidic manipulation, and they exploited the elastomeric properties of PDMS to dynamically and reversibly modify the cross section of the nanochannels; simply applying a few small weights on an elastomeric device, they succeeded in blocking a single DNA molecule inside a nanochannel.

Here, we propose an approach, based on soft lithography techniques, for the fabrication of elastomeric Lab-On-a-Chip (LOC) systems, which can be used for the separation of long DNA molecules, (longer than 30 kbp). The design of our device is composed of an array of nanochannels which can be easily and reversibly deformed using a piezoelectric system integrated onto the device. These structures are suitable

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to perform long DNA molecules separation experiments based on the entropic recoil sieving mechanism. Thus, our goal is the implementation of an elastomeric entropic recoil device with tunable cross section.

Here we report our results concerning the fabrication process and preliminary tests on DNA chain elongation inside nanochannels.

2 Fabrication/Characterization of Elastomeric Nanofluidic Structures and Analysis of DNA Molecules Stretching

Nanostructures are fabricated using replica moulding techniques starting from a micromachined silicon master. A Focused Ion Beam (FIB) is used in order to pattern an arrays of nanochannels, of various dimensions and shapes, on a particular region of the silicon microfluidic structure. One of the main advanteges of replica molding approach is the possibility of replicating micro and nanofeatures in a single step. A SEM (Scanning Electron Microscope) image of the region on the silicon master, where the macrochannel is interrupted and where nanochannels are milled is reported in Fig. 1 a). A higher magnification SEM picture shows several 300 nm wide nanochannels.

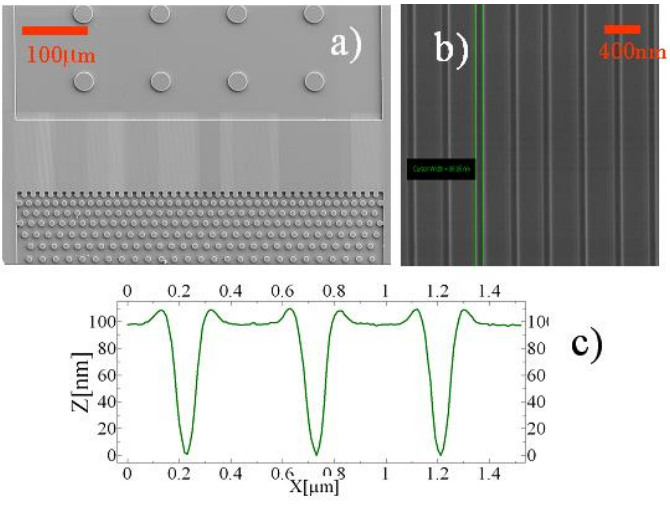

Fig. 1. a) SEM image of the microchannel region where nanochannels are patterned. b) SEM picture of FIB patterned nanochannels. c) Profile of three nanostructures acquired using an atomic force microscope.

Arrays of nanochannels of various width and depth are patterned in order to test different confinement conditions on DNA molecules, in particular width ranges from 350 to 600 nm and depth from 50 to 300nm. To characterize the nanofeatures of the silicon mold Atomic Force Microscopy is used, a profile of FIB nanofabricated structures is reported in Fig.1 c).

Before the replication of the silicon master using polymeric materials such as PDMS and h-PDMS, a thin anti stiction layer must be deposited on the stamp to reduce the silicon surface energy and favour the release of the replica from the mold.

To obtain an anti stiction layer of 1H,1H,2H,2H-perFluoroOctylTrichloroSilane (FOTS) molecules, vapour phase deposition is used, this process is generally called "silanization" because the molecules used to functionalize the surface are silane molecules. Before silanization, the silicon mold must be thoroughly cleaned in piranha solution and exposed to oxygen plasma. The structures on the mold are then replicated following a double replication process.

AFM images reported in Figure 2 demonstrate that the deposition of the anti stiction layer did not altered the reliefs patterned on the mold.

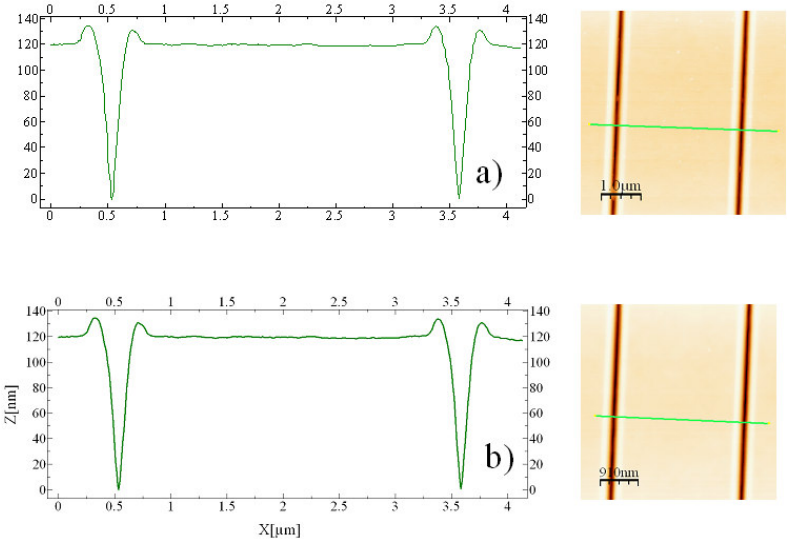

Fig. 2. AFM images and profiles of the silicon master, before a) and after b) the silanization, show no significant differeces, so a thin layer of vapor phase deposited FOTS molecules can be used as anti sticking layer as it does not alter the nanostructures on the mold

Replica molding consists in the deposition of two polymers which have different mechanical properties: h-PDMS, a material proposed by Schmid et al in 2000 [2], and PDMS (Sylgard 184, by Dow Corning). Thus, the replica results in a thin spin cast layer of stiff material (h-PDMS) and in a thick (3-4 mm) soft material (PDMS). In fact, from literature we know that h-PDMS has a Young's modulus which is nearly three times higher than Sylgard 184 (10:1, prepolymer:curing agent ratio).

As the first replica has negative features on its surface, it must be, in turn, replicated to obtain a polymeric replica with micro and nanostructures similar to the silicon mold ones. To verify the success of the replication procedure either at micro and nanoscale level we acquired images using optical and atomic force microscopy. Images of the fabricated positive h-PDMS/PDMS replica are reported in Fig 3.

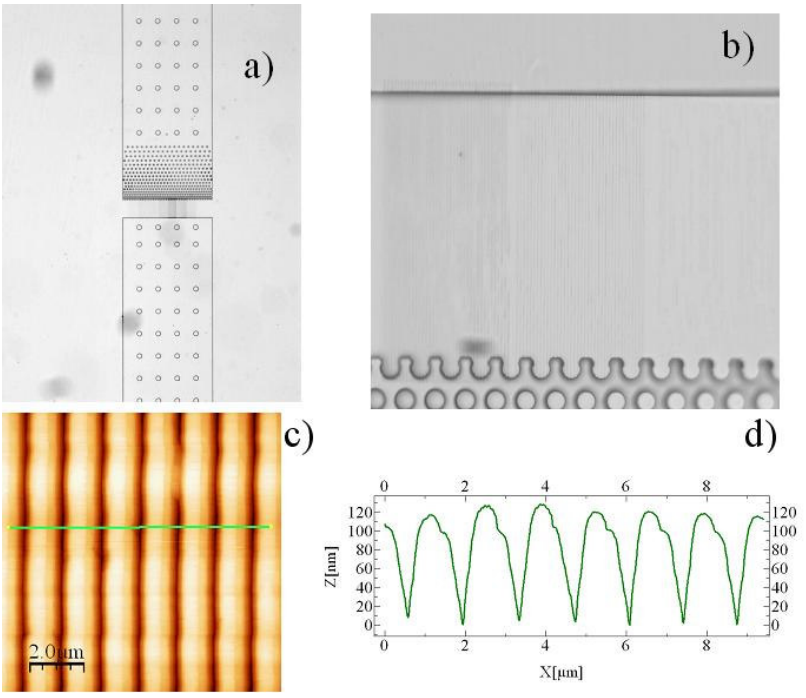

Fig. 3. a) 4X image of the positive h-PDMS/PDMS micro and nanochannels, b) 40X image of the nanochannels patterned on the positive replica. c) Atomic force microscopy image of an array of nanochannels patterned on the positive polymeric replica. d) The profile of the nanochannels reveals that they are nearly 120 nm deep.

AFM images confirmed the presence of nanochannels having various depth and width on the surface of the replica.

To confine fluids inside nanochannels, they must be irreversibly sealed; an effective and easy method to bond PDMS and other capping layers, such as glass cover slips or other thin layers of PDMS, is plasma oxidation; it is used to activate the surface of the polymeric replica in order to induce a covalent bond between the two surfaces. It is worth noting that the success of the bonding depends strongly on the flatness and on the cleanliness of the surfaces brought into contact. The use of h-PDMS resulted in being crucial at this point of the fabrication process, in fact due to its higher stiffness nanostructures presenting a thin layer of this material did not collapse after bonding, a problem that frequently affected positive replicas made only of PDMS.

The characterisation of the nanochannels after the sealing process is generally done taking advantage of capilary filling experiments. Just after the plasma oxidation PDMS, which has a hydrophobic nature, presents a hydrophilic behavior thus an acqueous solution of fluorescein (5 mM) can easily enter the micro and nanostructures because of capillary forces. Then, to verify if the nanochannels are open over their entire length (which is 100 μm), we observed, using an epifluorescence microscope, if

the solution entirely filled the nanochannels. As it can be seen in Fig. 4, using our soft lithography based approch, we were able to fabricate open nanostructures, in fact fluorescence signals were detected over the entire length of the channels.

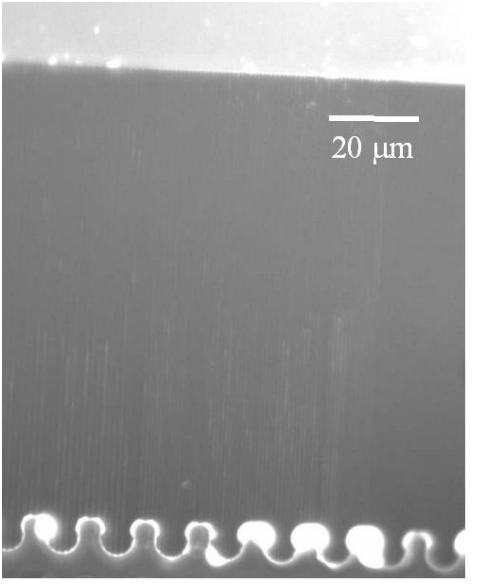

Fig. 4. An acqueous solution of fluorescein (5 mM) completely filled the nanochannels, it was used to demonstrate that they are open over their entire length

Preliminary tests on DNA behavior inside nanoconfined spaces were conducted. We used a solution of λ-DNA (48.5 kbp long) with a concentration of 0.1 μg/mL in tris-EDTA buffer; DNA molecules were stained with a dye-to-base pair ratio of 1:10 using an intercalating dye (YOYO-1, from Molecular Probes). The solution was inserted in the device and an epifluorescence microscope, with a CCD camera, was used to record DNA molecule stretching phenomena.

λ-DNA strands, in such a solution, should have a radius of gyration of nearly 1 μm [7], which is larger than the depth of the nanochannels fabricated on the h-PDMS/PDMS replica. From AFM images we found that nanochannels were from 100 to 170 nm deep, depending on the array considered, in fact on the positive replica several arrays of nanostructures having different sizes were present. Thus, DNA molecules must stretch and change their conformational state to pass through the confinement structures patterned on the device. The stretching depends on several parameters: persistence length and contour length of the chain and on the size of the confining structures. From our preliminary experiments on DNA molecule insertion in an elastomeric nanochannel we found that the mean length of the molecules was nearly 5 μm [8]. More tests are needed to confirm these observations.

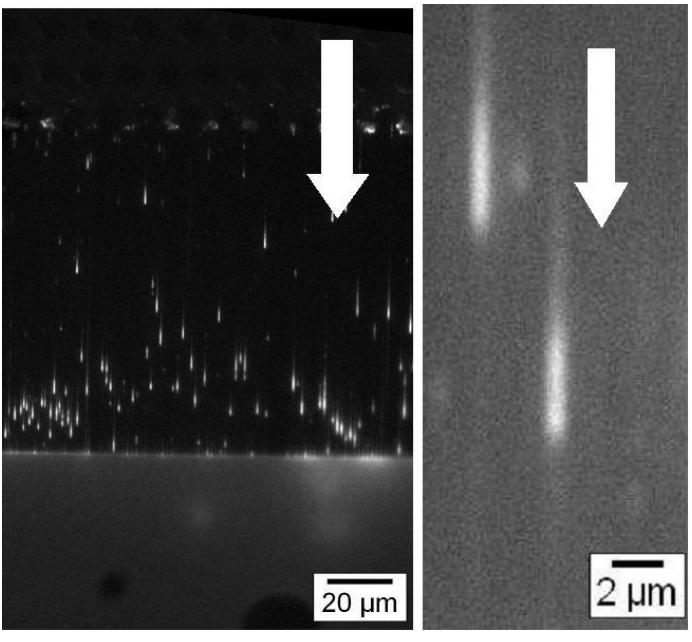

Fig. 5. Stayned λ-DNA molecules inserted in an array of nanochannels, from 100 to 170 nm deep and from 550 to 800 nm wide. The images were acquired using un upright epifluorescence microscope, for the picture on the left a 60X oil immersion objective was used, while the image on the right, which clearly shows two λ-DNA molecules, was captured using a 100X objective.

3 Conclusions and Perspectives

We proposed an effective method for the fabrication of elastomeric nanochannels whose dimensions and geometry can be easily tuned varying the FIB patterning parameters. We demonstrated that open nanochannels can be easily fabricated using h-PDMS, which, due to its high stiffness, is less affected by collapse problems than commerical PDMS.

We observed preliminary phenomena of DNA stretching on λ-DNA molecules inserted in elastomeric nanochannels, but these results must be confirmed.

Future perspectives of our work concern the application of pulsed electric fields to these nanostructures to implement "entropic recoil" based sieving. Moreover a piezoelectric system will be integrated in the polymeric device to dynamically deform the cross section of the nanostructures during the passage of DNA chains inside the nanochannels.

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