Uncoil long DNA molecules by nanoposts array

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ABSTRACT

A consistent and reproducible degree of stretching of DNA is important for single DNA molecule analysis such as DNA mapping and fast sequencing. Micro/nanopost or posts arrays are known to be able to uncoil genomic scale DNAs upon collision on the posts. Here, we built periodical hexagon nanoposts array embedded in a polymeric micro/nanofluidic device at low costs by a novel NanoImprinting process. The electrophoresis of λ-DNA and T4 DNA were studied in the posts array. A periodical stretching/recoiling motion was observed for both DNAs. Experiments showed a stretching to over 90% of its contour length was highly reproducible for both λ-DNA and T4 DNA. This non-gel electrophoresis of DNA also can be useful for the study of fundamental of DNA separation.

Keywords: DNA electrophoresis, DNA separation, nanoposts array, uncoil, entropic recoil

1 INTRODUCTION

The uncoiling of genomic DNA is one of the critical steps for DNA mapping, fast sequencing, and other single DNA molecule analysis. Though stretched DNAs can be found randomly on the wall of microfluidic chips, controllable uncoiling of DNA molecules has only been achieved by stretching with optical tweezers [1], confinement in nanochannel [2], and collision to micro/nanoposts [3-5].

Micro/nano posts are known for its ability to uncoil genomic scale DNAs [6], they are also shown capability to separate long DNA within less than 15 sec and length of 1 mm [4]. However, nanometer gap posts arrays with high density was hard to be achieved with conventional microfabrication methods. Therefore, the motion of large DNA molecule in a nanoscale posts array still unclear.

Here, we report the fabrication of hexagon nanoposts on PMMA chip by a novel Nano Imprinting (NIL) process. The nanopatterns was built on a silicon master stamp with high-end nanofabrication tools and then transferred to target PMMA substrate with 2-steps NanoImprinting process.

The motion of DNA molecules inside the posts array was studied. A periodic unravel-entangle-recoil process was observed under relatively low driving electric field. Upon entangle to the nanoposts, a stretching to over 90% of its contour length is highly reproducible for both λ-DNA and T4 DNA.

2 EXPERIMENTS

2.1 Chip fabrication

A two-step imprinting process was applied for the fabrication of the nanoposts array and micro/nanofluidic system. Fig.1 shows the schematic fabrication processes focused on the cross-section of the nanoposts area. First a silicon master (Fig 1a) with all the micro/nano scale fluidic structures was achieved. In addition to the photolithography-based dry/wet etching for micron patterns, nanoposts and nanochannel was build by Focused Ion Beam (FIB) milling. After coating a monolayer 1H,1H,2H,2H-perfluorodecyltrichlorosilane as anti-adhesion agent on silicon master, a drop of UV curable resin was dispensed on the master stamp (Fig 1b) and an oxygen plasma activated COC sheet was placed on for the substrate of UV resin (Fig 1c). After exposing UV light, the cross-linked UV resin was demolded from silicon master and served as a negative stamp (Fig 1d). Upon thermal Imprinting with the UV resin stamp (Fig 1e), positive patterns were transferred to target PMMA substrate. The patterned PMMA was then bonded with a thin PMMA cover cap to seal the micro/nanofluidic system. For more details about the fabrication and pattern transfer fidelity please review our previous work [7].

The size of nanoposts was decided by the parameter of FIB etching. The nanofluidic chips discussed in this paper have hexagon nanopost array with edge length of 150~400 nm, gap between posts of 70~350 nm, and height of posts of 70~350 nm.

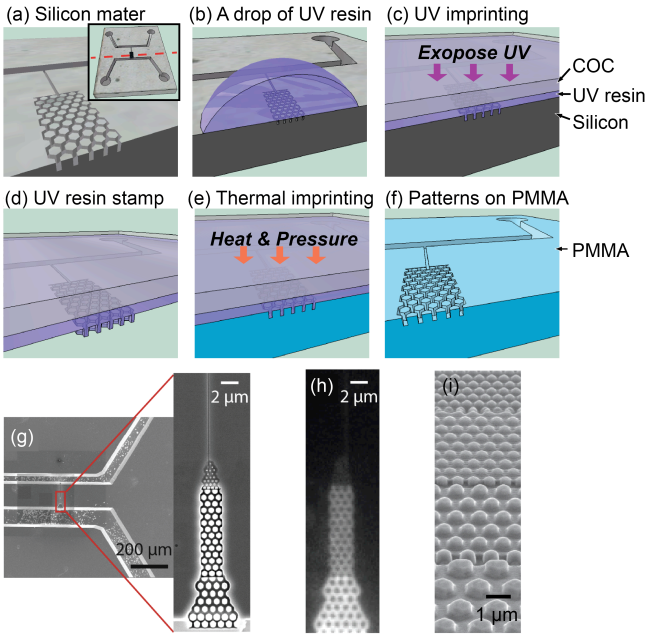

Fig 1. Schematic cross-section images of the fabrication flow. (a)~(c): Pattern transfer from Silicon master to UV resin stamp by UV-NIL, inset: view of the whole chip, dashed line stands for the position for taking the cross-section. (d)~(f): Pattern transfer from UV resin stamp to target PMMA substrate. (g): SEM images of nanoposts and nanochannel on PMMA, and (h): fluorescence image of sealed chip filled with FITC dyed buffer. (i) Enlarged SEM image of nanoposts in various dimensions taken with an angle of 52°

2.2 DNA translocation experiments

0.5 μg/ml λ-DNA and T4 DNA solution was prepared in 1x TBE buffer (89 mM Tris–borate and 2 mM EDTA, pH 8.3). DNA was dyed by YOYO-1 in 10:1 molar ratio(bp/dye). 3% β -Mercaptoethanol was added as anti-photobleaching agent, an enzymatic oxygen scavenger system consisted of 0.2 mg/mL glucose oxidase, 0.04 mg/mL catalase and 4 mg/mL β-D-glucose was also added in the buffer to remove oxygen radicals. A vacuum pump was used to drive the DNA solution together with the capillary force. A DC bias of 0.1~10 V was added by the Ag/AgCl wires in the reservoirs.

A fluorescence microscope and EMCCD system (Axiovert 200 M, Carl Zeiss, Thornwood, NY), which was equipped with a 100 /1.3 NA oil immersion objective (Carl Zeiss) and an EMCCD (PhotonMax 512B, Princeton Instruments, Trenton, NJ) was used for FITC and DNA imaging. The video was recorded at 20 ms/frame at overlap mode, and was analyzed by imageJ and a customized C program for the DNA motion study.

3 RESULTS

When confined in a nanopost array and driven by electric field, the motion of large DNA molecules consisted of periodical stretching and recoiling due to entangling to posts and unhooked from them respectively. The motion in one period can be roughly divided into three phases: 1) When the molecule collided to a post, two arms of the molecule chain threaded different from each side of the post, the molecule unraveled upon entangling to the post, 2) As the unraveled molecule reach its contour length, it was followed by a unhooking process where one arm of the chain moved along the electric field while the other arm of chain was dragged moved oppose to the electric field; 3) When the whole molecule was unwrapped from the nanopost, the tension built upon the chain and the entropy recoiled the molecule and prepared for the next collision.

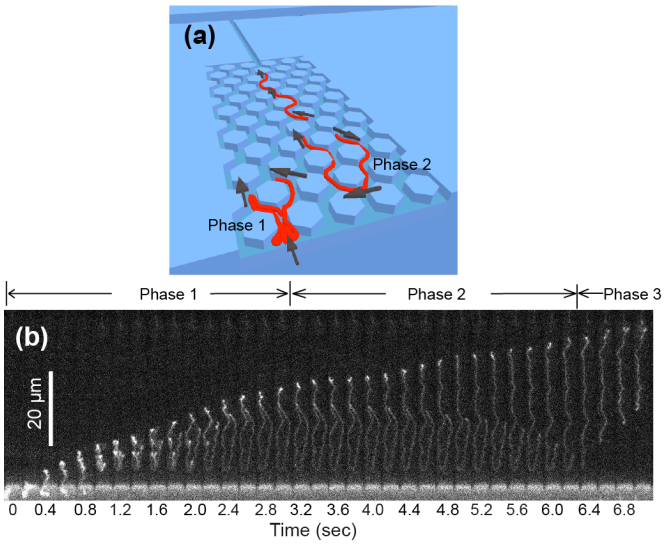

Fig 2a. The schematic of uncoiling DNA by nanopost array. The uncoiling process can be divided into three phases: 1. Unravel, 2. Unhook and 3. Recoil. The arrows in the images suggest motion direction of the DNA chain. Fig 2b. Captured video frames of a T4 DNA stretched by nanopillar array.

Phase 1 started from the DNA entangled to a nanoposts, and ended when one or both of the arms of the molecule chain were fully stretched. At the beginning of Phase 1, since the gap between the posts (500nm) is less than the gyration diameter of DNA (~880 nm for λ DNA and ~1.85μm for T4 DNA), the confinement from the post array geometry forced the DNA to deform from the sphere shape. The elongation of DNA in the array can be estimated by de Gennes theory: $L_z = L_{contour}\frac{(pw_{eff})^{1/3}}{D^{2/3}}$, here $L_{contour}$ is the contour length of DNA, p is the persistence length, w_{eff} is the effective width, and D is the size the gap between posts.

As the elongated molecule colliding to a nanoposts, very likely the head and tail of the molecule would thread into separate ways around the nanopost. The two arms of the molecule chain threads along various paths ever since. For long DNAs such as T4 DNA, the size of the divided parts was still large enough to be divided into more parts again. So, at the beginning of Phase 1, it's common to see that more than 3 arms of DNA chains were heading into the array via different paths, as it happened at 1.0 sec ~ 1.6 sec in Fig 2b. While the branches of chain threaded deeper into the array, the center of molecule still entangled to the nanoposts. The stress along the molecule chain therefore unfolded the coiled molecule, as showed in Fig 2. At the end of the Phase 1, only two arms existed, the shorter one was fully extended.

Phase 2 started from the fully stretch of short arm, and ends when the length of that arm is zero. In the beginning of Phase 2, the short arm was fully stretched, so the inner tension from DNA chain was not negligible anymore.

Almost all the collision events we observed are J collision, meaning that the two arms of the chain are in different length. Most likely, as it happened in Fig 2b, the long arm was not fully uncoiled when the short arm did.

After the fully uncoiling of the long arm, the system can be defined by the classical rope over pulley model. It was governed by

$$l_S + l_L = l_{contour}$$

$$(l_L - l_S)\rho E = l_{contour}\frac{\rho l_L}{dt}$$

it can be solved analytically,

$$l_L = \frac{1}{2}(l_{contour} + l_{\Delta 0}\exp(\frac{2E\mu t}{l_{contour}}))$$

$$l_S = \frac{1}{2}(l_{contour} - l_{\Delta 0}\exp(\frac{2E\mu t}{l_{contour}}))$$

$$l_\Delta = l_{\Delta 0}\exp(\frac{2E\mu t}{l_{contour}})$$

The unhooking time therefore can be estimated for each collision by

$$t_{unhook} = \frac{l_{contour}}{2\mu E}\ln(1 - \frac{2l_s}{l_{contour}})$$

At the end of Phase 2, the tail of molecule chain had passed through the entangled nanoposts. Therefore the inner tension of the molecule chain can't be balanced any more. Meanwhile, direction of electric field was also the same as the motion direction of the tail of the molecule chain right now. Therefore, in the beginning of Phase 3, once passed the entangled nanopost, the tail of the molecule chain moved faster than the head of the molecule. The length of the molecule decreased and the molecule coiled up again.

As the coiled molecule moving along the post array under the electric field, collision could happen. If the head and tail of molecule, or two segments of molecule threaded toward various ways around the collided nanopost, the molecule was tangled to the post again, and a new Phase 1 began.

Not all the DNAs were following exactly as the 3 Phase motion. We observed that for T4 DNA, sometimes in Phase 2, the long arm could collide on a post and divided into two new arms, while the short arm was still entangled to another post. However, even this can be understood as a sub-period inside one period of collision. Due to the shorter chain length of λ-DNA, this phenomenon was rarely seen on λ-DNA.

4 CONCLUSION

In this paper, we report the fabrication of hexagon nanoposts on PMMA chip by a novel Nano Imprinting (NIL) process. The nanopatterns was built on a silicon master stamp with high-end nanofabrication tools and then transferred to target PMMA substrate with 2-steps NanoImprinting process.

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ACKNOWLEDGEMENTS

We would like to thank staff at the Center for Advanced Microstructures & Devices of LSU for their technical support. This research was supported by National Science Foundation (CBET-1067583) and the National Human Genome Research Institute (NHGRI) in National Institutes of Health. SP thanks the Korea Research Foundation and The Korean Federation of Science and Technology Societies Grant funded by Korea Government (MOEHRD), Basic Research Promotion Fund for supporting this work.

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