Manipulating DNA Molecules in Nanofluidic Channels and Observing with Optical Molecular Imaging*

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Abstract λ-phage DNA molecules, stained with YOYO-1 fluorescent dye, were put inside sub-100 nm open channels which have width ~40 nm and depth ~60 nm. The DNA molecules were transported along these nanoconduits and the removing information was observed by fluorescence microscopy technique clearly. The movements of DNA molecules in the channels were discussed. Associating with optical molecular imaging, these scale nanostructured fluidic channels would be useful for studying single biomolecules statics as well as the dynamics.

Keywords Optical molecular imaging; DNA molecules; Nanofluidic Channels; Nanopore

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0 Introduction

The study of DNA molecules has been made great progress in the last two decades at the single molecule level with fluorescence microscopy[1,2]. Recently, with advancements of nanobiotechnologies, many different areas of research scientists including both fundamental study and applied techniques are affirmative focusing on the creating novel biophysics methods and nanoscale fluidic devices and using them on the study of single bio-molecules[3,4]. Especially, the process and characteristics of single bio-molecule transporting through a nanopore are fascinating more and more multidisciplinary scientists, and they expect to use nanoscale pore as a detector and analyzer to super-quickly sequence gene[5,6]. The process of DNA molecules perforation is consisted of several steps. It is obviously the initial step that manipulating a DNA molecule to move near and into the nanopore. Prevalent experiments are driving DNA molecules through nanopore stochastically. This approach is vague and uncontrolled.

A double-stranded DNA molecules diameter is only about 2.3 nm and its radius of gyration about ~60 nm and the persistence length ~50 nm in aqueous buffer solutions. It will be available to employ sub-100 nm fluidic channel arrays for manipulating DNA molecules and inducing them into a single nanometer pore subsequently. This process can be easily revealed in time by fluorescence microscopy techniques.

In this paper, it was introduced the information that it was observed single λ-phage DNA molecules transferring along nanofluidic conduits with fluorescence microscopy; the DNA molecules are stained with YOYO-1 dye, which is one effective fluorescent reagent. It was used focused ion beam milling techniques to fabricate high-resolution nanoscale fluidic conduit which width is ~40 nm and height ~60 nm.

1 Nano-scale fluidic channels fabrication

There are many reports of micro- and submicro-fluidic channel arrays in researches about single bio-molecules properties for many years. On the other hand, applications with sub100 nanometer and even single nanometer fluidic channel arrays in biomolecules and life science study were rarely reported. This limitation is mainly due to difficulty of fabrication process and metrology. Conventional techniques of fabricating nanoscale devices are soft X-ray or extreme-UV lithography, and electron beam lithography[7]. All these fabrication processes have deficiencies, such as susceptible to the processing environment, and

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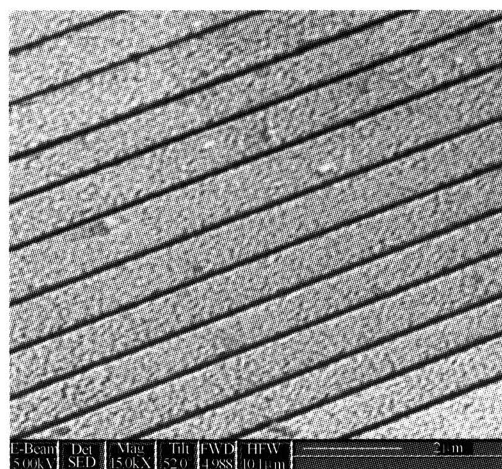
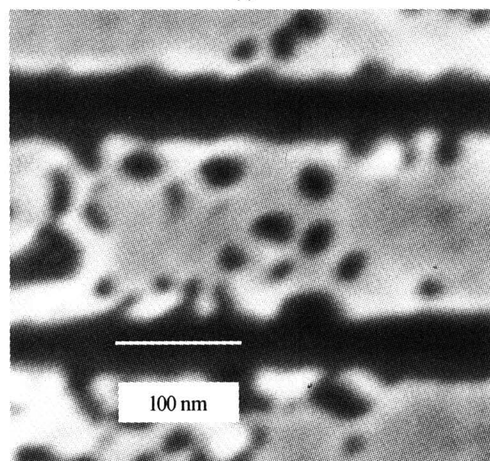
the creating pattern could not be complex, et al. Focused-ion-beam (FIB) tools are the first equipments that were specifically designed to image features on lithographic masks and to repair defects to produce a defect-free mask. It has been used to direct writing of three-dimensional structures on nanoscope level. In theory, ion beams can be focused to smaller dimensions than the beam resolution achieved by commercial SEM. FIB milling tools have a lot of advantages in nanoscale fabrication[8]. It is a practical solution to address the issue of fabricating nanofluidic devices fleetly and exactly according to the multifarious necessity.

Fig. 1 is a typical nano-channel arrays created by FIB on the surface of silicon nitride crystal membrane (Si_3N_4). About the nanofluidic conduit arrays, depth of almost all channels were about 60 nm, width about 40 nm, and length 50 μm. Si_3N_4 was chosen not only because it is a commonly used excellent dielectric material that scientists selected to fabricate synthetic robust nanopore, but also because



(a)



(b)

Fig. 1 SEM images of Nanofluidic channel arrays created by FIB, (b) is the local magnification of (a)

of its optical clarity and low auto - fluorescent background that will facilitate fluorescent microscopy experiments.

2 Materials and Experiments

2.1 λ-phage DNA molecules preparing and staining

Lambda DNA (λ-phage DNA) is a linear double-stranded helix that contains 48,502 bp, its molecular mass is ~30.6 MDa, and contour length is 16.2 μm[9]. It is widely used in life science study. In experiments, to prepare DNA molecules for fluorescence microscopy, λ-phage DNA (Sino-American Biotechnology Company, China) was stained with the intercalating fluorescent dye YOYO-1 (Molecular probes, M. W. 1270.65, oxazole yellow dimmer). In all the experiments, DNA base pair-to-dye ratio is kept at 10 : 1 (bp/dye=10). The final DNA concentration was 1 μg/mL in buffer containing 10 mM Tris-HCl, 10 mM NaCl and 1 mM EDTA, pH8.0. The DNA/YOYO-1 solution was incubated for ~30 min in a dark room, and then was diluted to 6.5 pM in a 50 mM Bis-Tris buffer (pH7.5, Sigma).

2.2 Fluorescence microscopy observing DNA molecules

Fluorescence microscopy is one basic, practical and useful technique in studying single DNA molecules[10,11]. With the development of high affinity intercalating DNA stains such as TOTO and YOYO, it has become one widely approved way to directly measure the length of single molecules by quantitating fluorescence. The amount of intercalated dye is proportional to the length of the molecule, so measuring the total fluorescent intensity from a single molecule gives a direct measurement of its length.

In the experiments, the stained λ-DNA molecules were illuminated by a 100 W mercury arc lamp in combination with a U-MWB excitation cube (BP450-480, DM500, BA515), and the DNA molecule were observed with an inverted optical microscope (IX-70, Olympus) by epi-fluorescence microscope equipped with a 20 × objective. Fluorescence images were obtained using a cooled charge-coupled device camera (CoolSNAP-fx, 1300 × 1030 pixels, 12-bit digitization, Roper Scientific, Inc.), the CCD acquisition time used is 2 seconds. YOYO-1 has an excitation maximum at 491nm and emission maximum at 509 nm. Fig. 2 is the sketching experiment setup of observing DNA molecules transporting along nanoconduits.

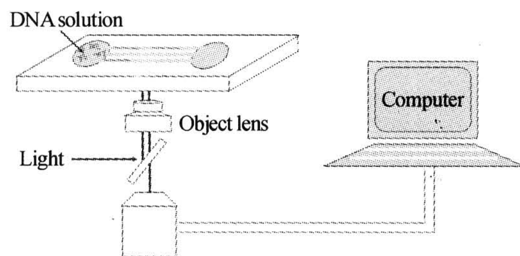


Fig. 2 Sketching map of experimental setup

2.3 Results and discussion

Silicon nitride is hydrophobicity in order to guarantee that the nanoscale fluidic conduits surfaces are hydrophilic, it washed the conduits using a solution mixture of Brij : H_2O=10g:50mL ratio about 20 min at room temperature before adding DNA solution. Brij ($C_{12}H_{25}(OCH_2CH_2)_{23}OH$) is one active reagent. Then, that DNA molecules were transported into open nanofluidic channels by capillary force was observed. Fig. 3 shows a representative fluorescence frame capture for λ-phage DNA passing along the open nanochannels. Scale bar for the image is 10 μm.

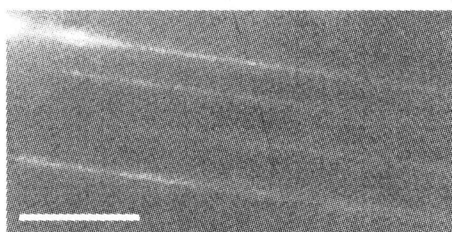


Fig. 3 A typical fluorescent image reveals the traveling of λ-phage DNA along the nanofluidic conduits

λ-phage DNA molecules passing along nanofluidic channels showed some newfangled but really amazed phenomena. It can be seen that DNA molecules could be stretched and threaded along open nano-channels, and molecules were moving all along the conduits interiorly, although they moved only a short distance rather than the whole channel. In addition, it can be seen that there were DNA molecules within some conduits and there were only buffer liquid within some conduits (this case is occasional), i.e. it was not all the conduits filled with DNA solution.

The information of DNA removing along nanochannels such as distance and velocity is involved with lots of parameters, for example, buffer concentration, surface tension, ion strength, and channel diameter, et al. It is known that if λ-DNA molecules flow through channels with a cross section much large than the size of the random coil, it is no doubt that the DNA molecules can pass entirely through the channel while preserving its random coil configuration. But, if λ-DNA is forced to flow through a nanofluidic channel with a crossed section comparable to the persistence length of the molecule, it is energetically more favorable for the DNA molecules to be in the stretched states. In this case, the bending of DNA to form a loop would cost energy much higher than the thermal energy. In our condition, DNA molecules were forced to remove along the open channels, the forces endured by DNA molecules were more complicated than that described above where they were transferred through a surrounded channel. Because the upper side of the conduit is open to air, DNA molecules not only affected by the force from the three solid walls (wall effect) of the conduit but also affected by the surface tension of the liquid-air interface. Fig. 4 is the sketch map to illustrate different cases of λ-phage DNA traversing along about ~50 nm diameter fluidic conduit array.

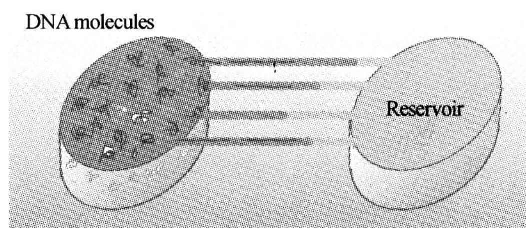


Fig. 4 Sketch map illustrating the traveling of λ-DNA molecules along the nanofluidic conduits

In experiments, DNA molecules were not transported through the whole channels, the reason is that the buffer solution could not been drilled through the open channels by capillary force[12]. Another reason may be that a DNA molecule block the entrance of conduit, in this case, itself or/and posterior DNA molecules could not be migrated. Fig. 5 is typical atomic force microscopy (SPA300HV-DFM (dynamic force mode), HIROX, JAPAN) images that illustrate the open conduits were blocked by DNA molecules, although the pattern is make from a wider fluidic channel. The configuration of DNA near the entrance of nanochannel is very important for its movement along the nanochannel. The coiled DNA molecule, or one entangled with channel wall or other molecules can't be entered the nanochannel, DNA molecules in the reservoir are not uncoiled molecules. The location of DNA molecule in the across section of the conduit (amid the solution or near or on the surface) is another important factor that influenced the DNA molecule moving, some DNA molecules maybe stuck to the surfaces of the conduit and finally blocked the conduit.

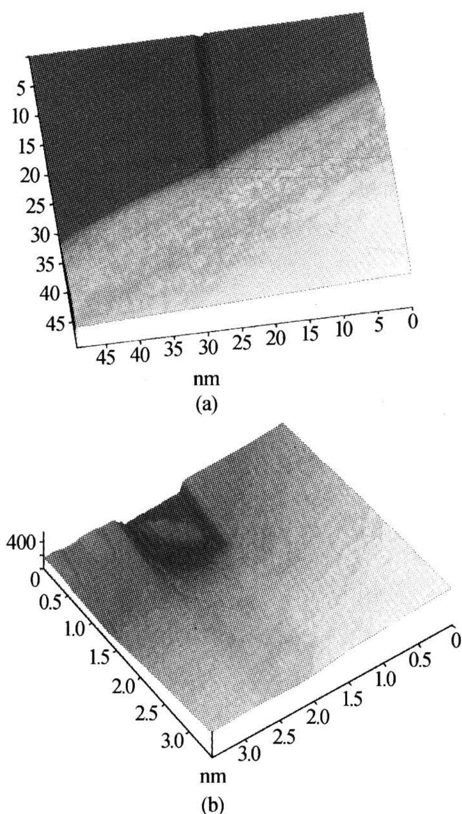


Fig. 5　AFM images of the fluidic conduit was blocked, (b) is the local Magnification of (a)

3 Conclusion

Single DNA molecules transferring along nanofluidic conduit arrays (conduit: ~ 40 nm width and 60 nm height) with optical molecular imaging were observed. Depending on YOYO-1 fluorescence, the preliminary study demonstrated that DNA molecules can not only be stretched within these sub-100 nm fluidic conduits entirely but also be transported along the channels. Because of the dimension of this fluidic conduit is close to natural state DNA molecule radius of gyration and persistence length in aqueous buffer solutions, this method would provide a promising way for detecting and analyzing single DNA molecules statics properties as well as dynamics. Moreover, it has other advantages, such as reducing the DNA sample amount, cutting down cost and time consuming. It is potential to integrate this scale of nanofluidic conduits into a biochip to study single DNA-molecules transporting through a nanopore as it can manipulate uncoiled nucleic acid entering nanopore.

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光学分子成像技术观察纳流芯片对DNA分子的研究

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摘 要 将λ-DNA与荧光染料YOYO-1结合,利用荧光显微技术对DNA分子在毛细现象作用下进入宽40 nm、深60 nm的纳米沟道内,并在其内部被拉伸以及沿沟道移动的情形进行了观察.讨论了DNA分子在沟道内的运动情况.结合光学分子成像技术与该尺寸范围的纳流芯片,将有助于研究生物分子的动力学和静力学性质.

关键词 光学分子成像,DNA分子,纳流芯片,纳米孔

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