Behavior of single DNA molecules in the well-ordered nanopores

Jau-Ye Shiu[a], Wha-Tzong Whang[a,*], Peilin Chen[b,*]

[a] Department of Material Science and Engineering, National Chiao Tung University, Hsin Chu 300, Taiwan
[b] Research Center for Applied Sciences, Academia Sinica, 128 Section 2, Academia Road, Nankang, Taipei 115, Taiwan

ARTICLE INFO

ABSTRACT

Here we describe a simple approach to fabricate robust three-dimensional periodic porous nanostructures inside the microchannels. In this approach, the colloidal crystals were first grown inside the microchannel using an evaporation-assisted self-assembly process. Then the void spaces among the colloidal crystals were filled with epoxy-based negative tone photoresist. After subsequent development and nanoparticle removal, the well-ordered nanoporous structures inside the microchannel could be fabricated. Depending on the size of the colloidal nanoparticles, periodic porous nanostructures inside the microchannels with cavity size of 330 and 570 nm have been obtained. The dimensions of interconnecting pores for these cavities were around 40 and 64 nm, respectively. The behavior of single λ-phage DNA molecules in these nanoporous structures was studied using fluorescence microscopy. It was found that the length of DNA molecules oscillated in the nanoporous structures. The measured length for λ-phage DNA was larger in the 330 nm cavity than those measured in the 570 nm cavity.

1. Introduction

Gel electrophoresis has been proven to be a very useful tool for separating biomolecules. However, the extension of gel electrophoresis for the separation of larger biomolecules was found to be problematic. To separate large biomolecules, there have been increasing research activities in developing nanofluidic systems where the dimensions of biomolecules are larger than the dimension of nanofluidic system. To optimize the separation efficiency of the nanofluidic system, it is necessary to understand the influence of geometric parameters in the nanofluidic system, such as channel dimension and geometry, to the biomolecules. Therefore, it is very important to investigate the behavior of single DNA molecules in various nanofluidic systems [1–4]. To construct nanofluidic systems, MEMs (micro-electromechanical systems)-based fabrication techniques are often used. One- and two-dimensional nanofluidic channels have been fabricated on the silicon-based substrates for the separation of DNA and protein molecules, for example, two-dimensional micron scale obstacles were integrated into microfluidic channels allowing the separation of DNA molecules [5–7]. Larger DNA or protein molecules could be also separated by nanofluidic devices consisted of entropic traps [8–11]. However, these fabrication techniques often required the use of sophisticated

lithographic techniques and the access to the clean room, which may deter many researchers in the field. Self-assembly of colloidal particles, on the other hands, is an alternative approach to construct well-ordered nanostructures without the access to the conventional lithographic tools where the close packed colloidal particles can be used as templates to fabricate various types of nanostructures. It has been shown by Colvin and coworkers [12] that three-dimensional periodic nanostructures could be produced by the self-assembly process of colloidal particles using capillary force where the size of these periodic nanostructures can be tuned using colloidal nanoparticles with different diameters. This approach was later modified by Ozin and coworker using micropatterns where the evaporation induced self-assembly process drove the colloidal particles into the pre-designed patterns forming colloidal crystals with controlled orientation [13]. Such approach allowed the construction of heterostructure in the microchannels [14] as well as the integration with the detection system [15].

Since the diameters of the colloidal nanoparticles are in the range of nanometer, the void spaces between nanoparticles form nanofluidic channels, which have been used as the sieving matrix in the microfluidic system for the separation of DNA molecules [16]. Because the close-packed colloidal crystals offered well-ordered size-controlled nanofluidic system, they have been utilized to investigate the behavior of single DNA molecules in the confined spaces [17–19] as well as the separation of small dye molecules [20] and biomolecules [21]. Previously, we have developed an addressable microfluidic system to control the growth of colloidal crystals at any position inside one- or two-dimensional microfluidic sys-

* Corresponding authors. Tel.: +886 2 2789 8000; fax: +886 2 2782 6680.
E-mail addresses: wtwhang@cc.nctu.edu.tw (W.-T. Whang),
peilin@gate.sinica.edu.tw (P. Chen).
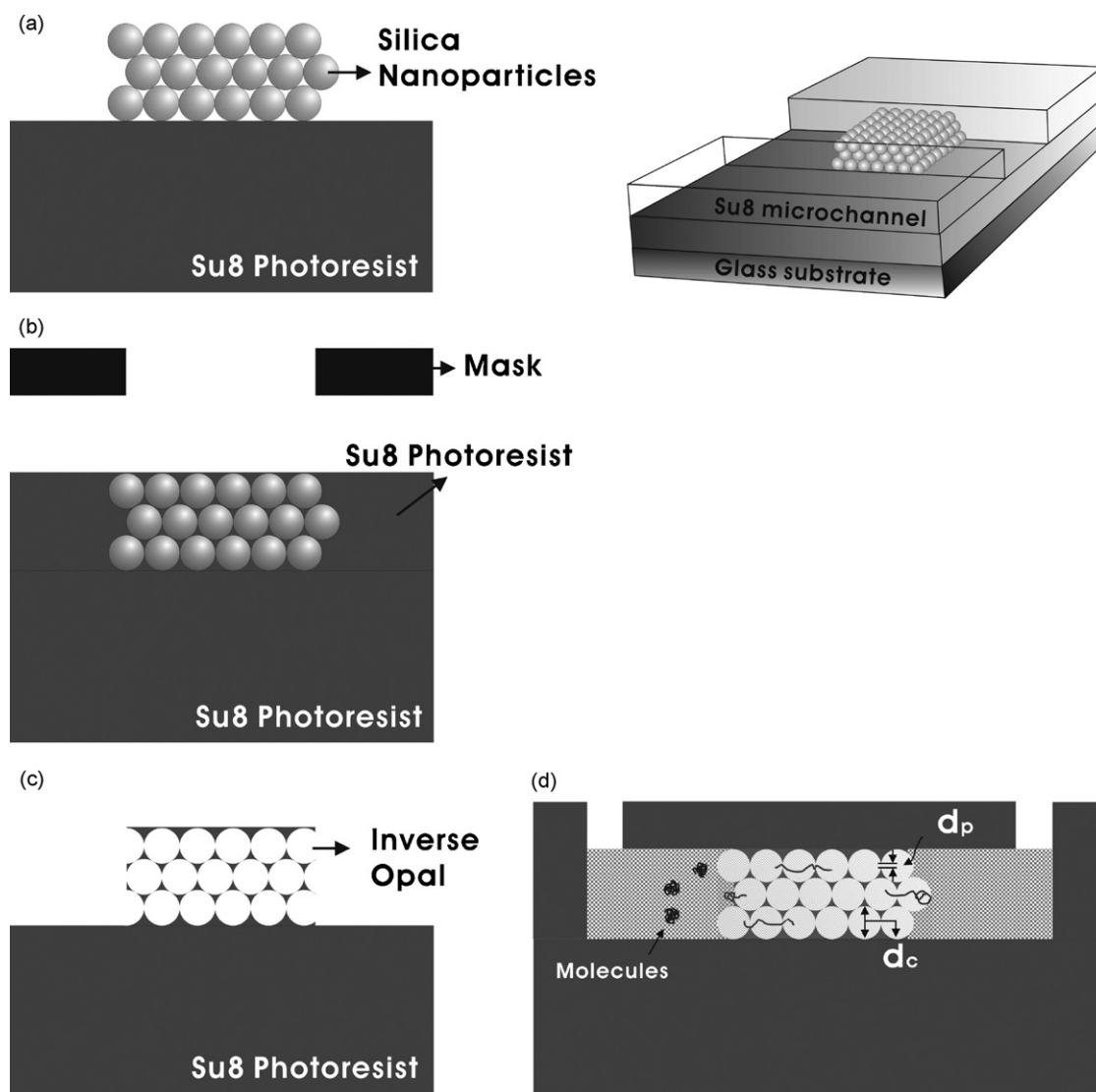
Fig. 1. Schematic for the fabrication of well-order nanoporous structure in the microchannel using SU-8 photoresist. (a) Silica colloidal crystals are first grown inside the SU-8 microchannel. (b) The void spaces of the colloidal crystals are filled with SU-8 photoresist and cured in the desired area using UV radiation. (c) Inverse opal structures can be obtained after removing the silica nanoparticles with BOE solution and sealing with another layer of SU-8 photoresist. (d) The nanoporous structures are consisted of cavity d_c and interconnecting pore d_p.

tem using electrocapillary effect [22,23]. Here, we present a similar approach to construct monolithically integrated periodic porous nanostructures in the microfluidic system using SU-8 photoresist (MicroChem, Newton, MA, USA) [24]. It is known that the cured SU-8 photoresist is highly resistant to acids and bases and they exhibit excellent mechanical properties and thermal stability. It has also been shown that the electrokinetic properties of SU-8 were similar to the commercial glass microdevices [25]. In addition, the photo patternable property of the photoresist would allow fabricating nanostructures at any desired location inside the microfluidic system. Therefore, it is advantageous to use SU-8 to construct nanofluidic system. In our approach, the SU-8 photoresist was used to fill up the void space inside the colloidal crystals. Upon the removal of the colloidal nanoparticles, the SU-8 photoresist formed an inverse structure of the colloidal crystals where cavities with diameter of the original colloidal nanoparticles as well as interconnecting nanopores could be obtained. These interconnecting nanopores were then served as sieving materials for the separation of biomolecules. The behavior of single DNA molecules was investigated in the nanofluidic system formed by the intercon-

necting nanopores as a function of applied electric field and cavity size.

2. Materials and methods

2.1. Chip fabrication

The size-tunable nanoporous chips were fabricated by a combination of photolithography and self-assembly of colloidal crystal, as described previously [20]. The schematic for the chip fabrication is illustrated in Fig. 1. The close packed colloidal crystal was first grown inside the SU-8 microchannels using an evaporation induced self-assembly process (Fig. 1a). The void spaces in the colloidal crystal were then filled with SU-8 photoresist. A photomask was used to define the location of the nanoporous structures inside the microfluidic channel (Fig. 1b). After dissolving silica colloidal particles in buffer oxide etch (BOE) solution, well-ordered nanoporous structures inside the microfluidic system can be obtained (Fig. 1c). These nanoporous structures were consisted of cavities with a diameter of d_c, which represented the size of the original sil-
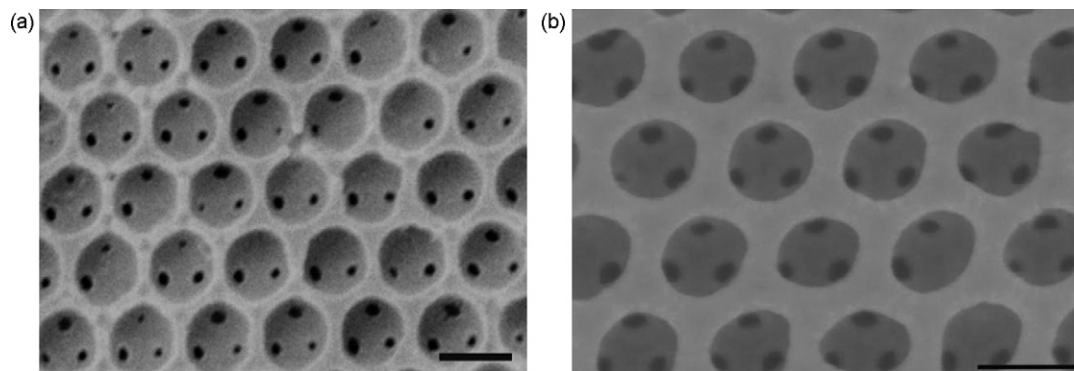
Fig. 2. SEM images of nanoporous structures. Silica nanoparticle size (a) 300 nm, bar: 300 nm; (b) 570 nm, bar: 500 nm.

ica nanoparticles, and interconnecting pores with a diameter of d_p (Fig. 1d). These interconnecting pores and cavities could be used as the sieving materials for separating biomolecules. The colloidal particles used in this experiment were 300 and 570 nm silica nanoparticles (Bangs Labs., Fisher, IN, USA).

2.2. DNA separation

To investigate the behavior of single DNA in the well-ordered nanoporous structure, λ-phage (MW: 48.5 kilo base pairs (kbp), Sigma) and M13mp18 (MW: 7.25 kbp, Sigma) DNA molecules were used. In this experiments, the DNA molecules were mixed with YOYO-1 dye (nucleic acid dye, trade name, Invitrogen, Carlsbad, CA, USA) at a ratio of 5:1 (base pair/dye) in 5× Tris borate EDTA buffer (TBE, pH 8.3, Sigma–Aldrich). An electrokinetic injection was used to introduce the DNA molecules to the sieving area. The fluorescence images of DNA molecules were taken on an Olympus IX71 inverted microscope equipped with a Cascade 512B CCD (charge coupled device) camera (Roper Scientific, Duluth, GA, USA).

3. Results and discussions

3.1. Monolithic integration of SU-8 microchannels

In this experiment, the three-dimensional ordered nanoporous structures were fabricated using SU-8 photoresist. There were two different dimensions in the well-ordered nanostructures: cavities (d_c), which were an inverse replica of the original silica nanoparticles, and interconnecting pores (d_p) where the close packed silica nanoparticles contacted each other. While the cavity size should be the same as the diameter of the silica nanoparticles, the diameter of the interconnecting pore was found to be about 10% of the diameter of the silica. The size tuning in this approach was achieved by varying the size of the silica nanoparticles. Shown in Fig. 2 are the cross-sectional scanning electron microscopy (SEM) images of two different nanoporous structures. The nanoporous structure formed by 300 nm silica nanoparticles is shown in Fig. 2a, while the nanostructure fabricated by 570 nm nanoparticle is depicted in Fig. 2b. From these SEM images, it can be clearly seen that the cavities were arranged in hcp (hexagonally close packed) close packed structures, which was the result of the self-assembly process. The cavity and interconnecting pore sizes were measured to be 297 and 40 nm when the 300 nm silica nanoparticles were used, whereas the colloidal crystals formed by 570 nm nanoparticles produced cavities with 575 nm diameter and 64 nm interconnecting pores. The dimension of the different nanostructures used in this experiment was summarized in Table 1.

Table 1
The relationship between the silica beads, cavity size and pore size

Silica particles size (nm)	Cavity size d_c (nm)	Pore size d_p (nm)
300	297 ± 8	40 ± 10
570	575 ± 10	64 ± 20

3.2. Behavior of individual DNA molecules

To see the influence of the nanostructure to the behavior of biomolecules in the nanofluidic system, we have investigated the behavior of single λ-phage DNA molecules in the nanoporous fluidic system at various applied d.c. electric fields. To observe the λ-phage DNA molecules in the nanoporous structure, the nanoporous structure was filled with 5× Tris borate EDTA buffer and the DNA molecules were labeled with fluorescence dye (YOYO-1) and the images of single DNA molecules were recorded by a fluorescence microscope (Olympus IX71) equipped with high speed camera (Cascade 512B EM CCD). Shown in Fig. 3 are the sequential images of λ-DNA molecules migrating inside the nanoporous structure toward the anode at an applied field of 5 V/cm. The time interval between each frame was 0.1 s. Two different sizes of nanoporous structures were used in this experiment, the cavity size were 300 nm (Fig. 3a) and 570 nm (Fig. 3b), respectively. Since the radius of gyration for λ-phage DNA molecules was larger than diameters of cavities and interconnecting pores in both cases, it was found that the λ-phage DNA molecules were stretched when they passed through these nanoporous structures. And the longest observed length was measured to be around 20 μm, which was about the contour length of λ-DNA molecules. Therefore, the λ-phage DNA molecules could be fully stretched in these nanoporous

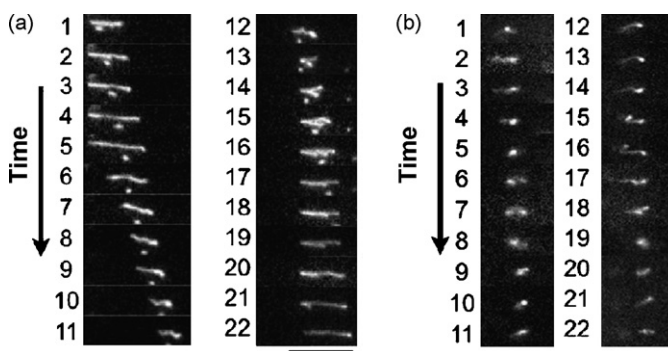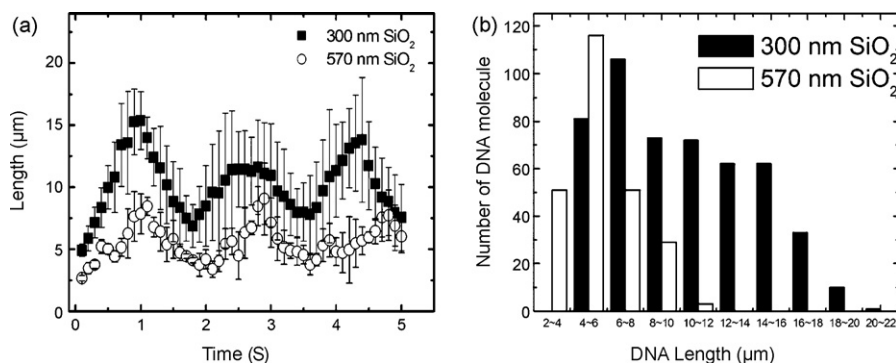**Fig. 3.** Sequential images of λ-DNA migrating inside the nanoporous structure toward the anode at an applied field of 5 V/cm. Elapsed time between frames is 0.1 s. Cavity size: (a) 300 nm, bar: 20 μm and (b) 570 nm; bar: 20 μm.

Fig. 4. (a) Averaged DNA length in two different nanoporous structures. Applied field: 5 V/cm. 300 nm (black square), 570 nm (open circles). (b) DNA length distribution in the nanoporous materials with two different cavity sizes (average of 500 DNA molecules).
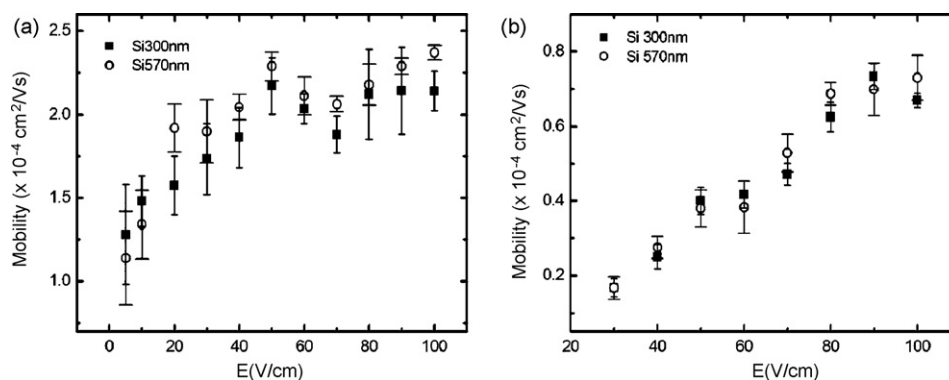
Fig. 5. The electrophoretic mobility of (a) λ-DNA and (b) M13mp18 vector as a function of the applied electric field with two different cavity size of nanoporous structures: 300 nm (black square), 570 nm (open circles) (average of 50 DNA molecules).

structures. Comparing with the averaged length of λ-phage DNA molecules measured in two different cavities, it was found that the length of DNA molecules measured in smaller cavity (300 nm) was larger than those measured in larger cavities (570 nm). In another word, the DNA molecules could recoil back to the coiled state more easily when they were in the larger cavities. The average length of the DNA molecules in two different cavities as a function of time was depicted in Fig. 4a. The time origin was randomly selected at the frame where the length of the DNA molecules was measured to be the smallest. The length distribution of DNA molecules in two different cavities is shown in Fig. 4b. It was found that the length of the DNA molecules oscillated with the same frequency in these nanoporous structures. However, the exact origin of this phenomenon was not known at this time.

3.3. Mobility measurement

To utilize the nanoporous sieving materials to separate the biomolecules, it is very important to measure the mobility of the biomolecules in these media. Shown in Fig. 5a is the measured electrophoretic mobility of λ-phage DNA molecules in two different sizes of cavities whereas the mobility of smaller M13mp18 DNA molecules in the same media was depicted in Fig. 5b. The electrophoretic mobility measured in the cavities formed by nanoparticles with a diameter of 300 nm was similar to those measured previously [24]. However, the mobility of both DNA molecules in the cavities formed by 570 nm colloidal particles was slightly higher at higher applied field. Since the sizes of the interconnecting pores in both cases were much smaller than the radius of gyration of both DNA molecules, we expect the entropic trapping effect to dominate in such type of sieving materials.

4. Conclusions

In summary, we have constructed well-ordered nanoporous structures inside the microfluidic channels using self-assembly process of colloidal nanoparticles. It was found that the cavity size of these nanoporous structures was the same as the diameter of the original colloidal nanoparticles whereas the size of the interconnecting pores was found to be about 10% of the cavity size. The influence of the nanostructures to the DNA molecules was measured on a single molecular level where the time dependent stretch-recoil behavior of the λ-phage DNA was recorded. The average length for λ-phage DNA molecules was found to be larger in the 300 nm cavity than those measured in the 570 nm cavity. The mobility of both λ-phage and M13mp18 DNA molecules was measured as a function of applied field. It was found that the electrophoretic mobility for the smaller M13mp18 DNA molecules was smaller than the much larger λ-phage DNA molecules, which indicated that the well-ordered nanoporous structures could be used to construct integrated nanofluidic system for the separation of large biomolecules.

References

[1] Y.C. Chan, Y.K. Lee, Y. Zohar, J. Micrimech. Microeng. 16 (2006) 699.
[2] J. Han, H.G. Craighead, J. Vac. Sci. Technol. A 17 (1999) 2142.
[3] J.T. Mannion, C.H. Reccius, J.D. Cross, H.G. Graighead, Biophys. J. 90 (2006) 4538.

[4] Y.W. Lin, M.F. Huang, H.T. Chang, Electrophoresis 26 (2005) 320.
[5] C.F. Chou, O. Bakajin, S.W.P. Turner, T.A.J. Duke, S.S. Chan, E.C. Cox, H.G. Craighead, R.H. Austin, Proc. Natl. Acad. Sci. U.S.A. 96 (1999) 13762.
[6] L.R. Huang, J.O. Tegenfeldt, J.J. Kraeft, J.C. Sturn, R.H. Austin, E.C. Cox, Nat. Biotechnol. 20 (2002) 1048.
[7] L.R. Huang, E.C. Cox, R.H. Austin, J.C. Sturm, Science 304 (2004) 987.
[8] J. Fu, P. Mao, J. Han, Appl. Phys. Lett. 87 (2005) 263902.
[9] J. Han, S.W. Turner, H.G. Craighead, Phys. Rev. Lett. 83 (1999) 1688.
[10] J. Fu, J. Yoo, J. Han, Phys. Rev. Lett. 97 (2006) 018103.
[11] J. Han, H.G. Craighead, Science 288 (2000) 1026.
[12] P. Jiang, J.F. Bertone, K.S. Hwang, V.L. Colvin, Chem. Mater. 11 (1999) 2132.
[13] S.M. Yang, G.A. Ozin, Chem. Commun. 24 (2000) 2507.
[14] S.M. Yang, H. Miguez, G.A. Ozin, Adv. Funct. Mater. 12 (2002) 425.
[15] G.A. Ozin, S.M. Yang, Adv. Funct. Mater. 11 (2001) 95.
[16] M. Tabuchi, M. Ueda, N. Kaji, Y. Yamasaki, Y. Nagasaki, K. Yoshikawa, K. Kataoka, Y. Baba, Nat. Biotechnol. 22 (2004) 337.
[17] D. Nykypanchuk, H.H. Strey, D.A. Hoagland, Science 297 (2002) 987.
[18] H. Zhang, M.J. Wirth, Anal. Chem. 77 (2005) 1237.
[19] Y. Zeng, D.J. Harrison, Electrophoresis 27 (2006) 3747.
[20] S. Zheng, E. Ross, M.A. Legg, M.J. Wirth, J. Am. Chem. Soc. 128 (2006) 9016.
[21] Y. Zeng, D.J. Harrison, Anal. Chem. 79 (2007) 2289.
[22] J.Y. Shiu, C.W. Kuo, P. Chen, J. Am. Chem. Soc. 126 (2004) 8096.
[23] J.Y. Shiu, P. Chen, Adv. Mater. 17 (2005) 1866.
[24] C.W. Kuo, J.Y. Shiu, K.H. Wei, P. Chen, J. Chromatogr. A 1162 (2007) 175.
[25] T. Sikanen, S. Tuomikoski, R.A. Ketola, R. Kostiainen, S. Franssila, T. Kotiaho, Lab Chip 5 (2005) 888.