Fabrication of long poly(dimethyl siloxane) nanochannels by replicating protein deposit from confined solution evaporation

Kuo-Feng Lo¹ and Yi-Je Juang^{1,2,3,a)}

¹*Department of Chemical Engineering, National Cheng Kung University,
No. 1 University Road, Tainan 70101, Taiwan*

²*Center for Micro/Nano Science and Technology, National Cheng Kung University,
No. 1 University Road, Tainan 70101, Taiwan*

³*Research Center for Energy Technology and Strategy, National Cheng Kung University,
No. 1 University Road, Tainan 70101, Taiwan*

(Received 6 April 2012; accepted 5 June 2012; published online 19 June 2012)

A relatively simple, inexpensive and reliable technique was developed to fabricate an array of nanochannels. Moreover, the nanochannels are directly integrated to microchannels as a whole, which facilitates solution loading from the millimeter-scaled loading reservoirs into the nanochannels. It is found that continuous bovine serum albumin (BSA) line structures with triangle-like cross section at nanoscale can be obtained by evaporation of BSA solution with concentration between 0.5 wt. % and 1 wt. % inside the microchannels. The poly(dimethyl siloxane) nanochannels were replicated from these line structures, followed by sealing with the glass slide. The DNA molecules can be stretched inside the nanochannels as fabricated. © 2012 American Institute of Physics. [<http://dx.doi.org/10.1063/1.4730371>]

I. INTRODUCTION

In recent years, study of nanofluidics has been attracting great attentions due to its intriguing transport phenomena at the nanoscale and potential breakthrough in fundamental knowledge. In addition, it not only is great tool to study single molecules^{1–4} but also has a wide range of applications ranging from ion transport, drug delivery to DNA, and protein manipulation.^{5–10} There are numerous techniques which can be used to fabricate nanochannels such as electron beam lithography (EBL), scanning probe lithography (SPL), nanoimprint lithography (NIL), and so on. Soft lithography is another commonly used technique for micro/nanofabrication owing to its simplicity, versatility, rapid prototyping, and low cost.¹¹ The core component for this technique is an elastomeric material, i.e., poly(dimethyl siloxane) (PDMS) and, with the aid of the PDMS, excellent replication of the micro/nanofeatures can be obtained readily for subsequent utility. Besides utilizing PDMS to replicate the nanostructures created by the conventional approaches, several novel strategies have been proposed and demonstrated, which are related to usage of PDMS. For example, a PDMS slab was exposed to oxygen plasma and stretched to generate linear nanoscale cracks, which were replicated onto ultraviolet-curable epoxy. The PDMS prepolymer is then cast against the epoxy mould to generate negative relief patterns of nanochannels, which were sealed with an oxidized PDMS slab to form the enclosed nanochannels.¹² The PDMS nanochannels can also be constructed by the elastomeric collapse of microchannels.¹³ Xu and Zhao used the core–sheath structures consisted of an electrospun polyvinylpyrrolidone (PVP) core and a sputtered aluminum sheath and embedded the structures in PDMS.¹⁴ The nanochannels were fabricated after the rupture of the sheath during master template and removal of the nanofibers. The nanofibers can be electrospun directly onto the

^{a)} Author to whom correspondence should be addressed. Electronic mail: yjjuang@mail.ncku.edu.tw. Tel.: +886 62757575 ext. 62653. Fax: +886 62344496.

silicon substrate and the nanochannels were obtained by casting PDMS prepolymer and being cured.¹⁵

Implementation of interconnect between macro to micro and micro to nano is also an important issue to ensure successful delivery of solution into the nanochannels. A review article can be found in the literature despite it emphasized on macro to micro interfaces.¹⁶ The interconnect between micro to nano can be fabricated directly¹⁷ or utilizing membranes with nano-scale through pores.^{18,19} In this study, we proposed a relatively simple and reliable technique to fabricate nanochannels with interconnect at microscale. Nanochannels with 70–120 nm in width

FIG. 1. The schematic of confined evaporation process for fabrication of PDMS nanochannel array. The protein (i.e., BSA) solution evaporates and the BSA molecules deposit inside microchannels to form line structures at nano/micro scale. PDMS nanochannels can be fabricated by replicating the line structures, followed by bonding the micro/nanogrooves onto glass substrate.

and over 5 mm in length were obtained *via* replicating the line structure of protein deposit, followed by sealing with glass substrate. Stretching DNA molecules was demonstrated inside the nanochannel as fabricated.

II. EXPERIMENTAL

A. Materials

Poly(dimethyl siloxane) (PDMS) (Sylgard 184) was purchased from Dow Corning. Bovine serum albumin (BSA) was purchased from Sigma-Aldrich. λ -DNA was purchased from New England BioLab and labeled with YOYO-1 (Y3601, purchased from Invitrogen) for observation.

B. Fabrication and characterization of nanochannels

Figure 1 shows the schematic of fabricating the PDMS nanochannels. The PDMS stamp having the microgrooves with approximately 100 μm in width, 4.5 μm in height, 1 cm in length, and 100 μm in spacing was fabricated via soft lithography. One end of the microgrooves was on the edge of the stamp. A droplet of BSA solution (with addition of fluorescein for visualization under the optical microscope) was first dispensed on a cleaned glass slide, followed by covering the PDMS stamp. Care needs to be taken to avoid the air bubbles trapped inside the microchannels. The protein solution was allowed to evaporate inside the microchannels at the ambient condition. The PDMS stamp was then removed and the BSA micro/nanostructures were obtained. The PDMS with 10:1 base-to-cross linking agent ratio was poured onto the BSA micro/nanostructures and the negative relief was obtained. It was subsequently treated with O_2 plasma and then bonded to the glass slide to form the micro/nanochannels. The optical microscope and scanning electron microscope (SEM) were used to characterize the micro/nanostructures and micro/nanochannels. The height of the micro/nanostructures was measured by atomic force microscope (AFM).

III. RESULTS AND DISCUSSION

It is found that complete filling of the PDMS microchannels with BSA solution will be affected by the channel dimensions. As the height of microchannels being larger than 4.5 μm , completely filling of solution becomes challenging and air bubbles will be trapped inside the confinement. For channels with low aspect ratio (e.g., 100 μm in width and 2 μm in height), the roof collapse will occur.^{13,21} Therefore, within the abovementioned constraints, one of the microchannel dimensions (100 μm in width, 4.5 μm in height, and 100 μm in spacing) was utilized in this study, and the BSA solution can readily fill almost all the microchannels without trapping air

FIG. 2. The images of BSA deposit for solution concentration (a) 0.5 wt. % and (b) 1 wt. %. (a1) and (b1) are the optical images. (a2) and (b2) are SEM images. (a3) and (b3) are AFM images with corresponding measurements (a4) and (b4). (The scale bar in the inset of SEM images is 100 nm.)

FIG. 3. The SEM images of BSA deposit at the bottom corners of and along the microchannel.

bubbles. Figure 2 shows the deposition of BSA molecules after solution evaporation. For 0.1 wt. % BSA solution, few straight lines can be observed under the optical microscope. The fluorescent intensity for most of the straight lines is either vague or non-uniform along the straight lines. This indicates that the solute content in 0.1 wt. % BSA solution is not sufficient to deposit and form continuous line structures after solution evaporation. As the concentration increases to 0.5 wt. %, all the straight lines can be observed under optical microscope and SEM as shown in Figures 2(a1) and 2(a2). Much more clear line pattern was obtained when the BSA concentration

FIG. 4. The height of the deposited BSA structures at different solution concentrations. The average heights of the deposited BSA structures from using 0.5 wt. %, 1 wt. %, 2 wt. %, and 3 wt. % solution are approximately 210, 530, 1030, and 1840 nm, respectively.

FIG. 5. The images of (a) the deposited BSA structures and (b) the negative relief near the open end of the microchannels (1 wt. % solution was used)

further increases up to 1 wt. % as shown in Figures 2(b1) and 2(b2). The average heights of the deposited BSA line structures are approximately 200 nm and 500 nm for 0.5 wt. % and 1 wt. % BSA solution used, respectively. When the BSA concentration increases over 6 wt. %, deposition of BSA molecules was also found at the bottom of the microchannel, which is no longer forming the line structures (data not shown) and is beyond the scope of the current study. Formation of the line pattern and uniformity of the line structures depend not only on the solution concentration but also the dimensions of the microchannels, their location away from the edge of the stamp and the surface property of the substrate as well.²⁰ Because of the slow evaporation process, the BSA molecules deposit at the bottom corner of the microchannels and the cross section of the line structures resembles a triangular shape as shown in Figure 3. The aspect ratio (the height to the bottom width) of the deposited structures is approximately 0.6 to 1 for concentrations lower than 3 wt. %.

Figure 4 shows the height of the line structures vs. BSA solution concentration. The data were acquired around the center of the line structures. It can be seen that the higher the solution concentration, the larger the height of the structures. Since the continuous line structures at nano and sub-micron scale can be obtained by evaporation of BSA solution with concentrations between 0.5 wt. % and 1 wt. %, this range of solution concentrations was used for fabrication of the PDMS nanochannels.

It is also found that there is substantial deposition of BSA molecules at the end of the microchannels which is open to the ambient as shown in Figure 5(a). Since the evaporation rate at the open end is much higher than that at other locations, it is hypothesized that the fluid flow

FIG. 6. (a) Top view and (b) 45° tilted view of the negative relief of the deposited BSA structures. (The scale bar in the inset is 1 μm)

FIG. 7. The nanochannels are filled with the 0.1 wt. % BSA solution from the inlet to the outlet. The solution was added with fluorescein dye for visualization. (“L” stands for the distance from the inlet)

toward the open end could be induced, which brings more BSA molecules to deposit at the open end. Figure 5(b) shows the negative relief where the integration of micro to nano is achieved. Figure 6 shows the PDMS nanogroove replicated from the BSA line structure obtained by evaporation of 1 wt. % BSA solution. Despite the wrinkle-like topography was observed on the PDMS surface, a clear and continuous nanogroove was obtained.

In order to verify that there is clear passage inside the nanochannels, 0.1 wt. % BSA solution added with fluorescein was loaded into the nanochannels and observed under the optical microscope. It was found that only a trace amount of BSA solution flowed into but did not fill the entire nanochannels fabricated from using 0.5 wt. % solution. It is speculated that the “channel annihilation” occurs inside the nanochannel which seals the flow passage.¹³ When using the nanochannels fabricated from 1 wt. % solution, the BSA solution filled into the channels from the inlet (micro to nano) to the outlet as shown in Figure 7. It can be seen that the microchannel (~ 1.5 – 2 mm) connects to two nanochannels with over 5 mm in length and $100\ \mu\text{m}$ in spacing.

The λ -DNA solution ($0.1\ \mu\text{g}/\text{ml}$) was directly loaded into the micro/nanochannel and the stretched DNA molecules were observed under the optical microscope as shown in Figure 8. Because of the capillary force and the integration of micro to nano scale, the DNA solution was readily introduced into the nanochannels. The stretched λ -DNA molecules were measured between 7 and $10\ \mu\text{m}$ in length, which is about 35%–50% extension of its full length. Since the elongation ratio (length of stretched molecule divided by its fully extended length, L_z/L) is proportional to $(pw)^{1/3}d^{2/3}$, where p is the persistence length, w is the width of the molecule, and

FIG. 8. Stretching of DNA molecules (a) near the inlet (micro to nano region) and (b) at about 2 mm away from the inlet of the nanochannel. The maximum elongation of λ -DNA reaches $10\ \mu\text{m}$, which is about 50% of its fully extended length. (Scale bar: $20\ \mu\text{m}$)

d is the width of nanochannel,⁵ the dimension of the nanochannel is estimated between 70 and 120 nm. It can be seen that there is reduction in the channel dimension when compared to the deposited BSA structures or its negative relief. This is attributed to both the triangular shape of the PDMS nanochannels and the adhesion force between PDMS and glass slide.^{12,13}

IV. CONCLUSIONS

In summary, we have developed a relatively simple, inexpensive and reliable technique to fabricate an array of nanochannels. Moreover, the nanochannels connected to microchannels are simultaneously constructed, which facilitates solution loading into nanochannels. It is found that continuous BSA line structures with triangle-shaped cross section at nanoscale can be obtained by evaporation of BSA solution with concentration between 0.5 wt. % and 1 wt. %. The PDMS nanochannels with approximately 100 nm in width and 1 cm in length were constructed by replicating the line structures deposited by 1 wt. % BSA solution, followed by bonding the negative relief with the glass slide. Stretching of DNA molecules was demonstrated inside the nanochannels as fabricated.

ACKNOWLEDGMENTS

This work was partially supported by National Science Council (NSC 96-2221-E-006-129).

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