Nanochannels for Manipulation of DNA Molecule using Various Fabrication Molecule

M.T. Hwang, Y.H. Cho, S.W. Lee, N. Takama, T. Fujii, and B.J. Kim

Abstract—**In this report, several fabrication techniques for the formation of various nanochannels (with SiO_2, Si, or Quartz) are introduced. Moreover, simple fabrication technique for generating SiO_2 nanochannels without nanolithography is presented. By using different nanochannels, the degree of stretching DNA molecule will be evaluated. Finally, we introduce a nanometer scale fluidic channel with electrodes on the sidewall of it, to detect and analyze single DNA molecule. The cross sectional shape of the nanotrench is V-groove, which was implemented by thermal oxidation. Electrodes were deposited through both sidewalls of nanotrench and the sealing of channel was done by covering thin poly-dimethiysiloxane (PDMS) polymer sheet.**

Index Terms—**Nanochannel, DNA stretching, LOCOS, thermal oxidation**

I. INTRODUCTION

Nanochannels have been taken an extensive attention recently since they provide powerful tools in manipulation and analysis of biomolecules, such as DNA and proteins. Nanochannels with dimension close to persistence length of DNA around 100 nm offers a proper condition for physical confinement or stretching on biomolecules, so that it provides many opportunities to reveal information of sample [1], [2]. The possible applications of the nanochannel with rendering DNA in stretched state consist of quick local mapping of DNA, DNA-protein interaction at single molecule level, and parallel analysis. For the validation and confirmation of these biological theory and phenomenon, the realization of nanochannel is crucial with micro and nanoscale hybrid patterning on devices, flexible in design, biocompatible with molecules and available in mass production [3]. So far, many of fabrication methods have already shown their capability of making nanochannels. A focused beam has been widely employed such as electron beam lithography and focused ion beam for they enable controllable 10-nm scale patterning easily [4], [5]. However, mass production is not feasible due to their serial processing nature and large area patterning is not adequate. Nano-imprinting lithography has been proposed as an alternative to solve these problems [6]. In nano-imprinting, a master is fabricated first generally with both e-beam lithography and optical lithography ways, and then the master mold pattern, which covers from nanoscale patterns to macroscopic area, is replicated in parallel process. However, fabrication is not flexible because a new master mold must be made whenever there is a change in design. The wearing of the mold may face the trouble for mass production. The usage of sacrificial layer was also suggested for the fabrication of nanochannel through differential etching techniques [7]. This technique is able to directly create sealed channel in one step. However, normally it takes long time to etch and sometimes suffers from the residual stress and mechanical defects of the capping layer. Sealing of etched structure to create channels is another issue in the fabrication. The sealing structure must be reliable and not intrude into the channel. Wafer bonding is a very reliable way to seal trenches. Anodic bonding is commonly used to bond a flat Pyrex glass on silicon with high electric field and heat [8]. Instead

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attaching an additional wafer on top of etched structures, direct sealing can be employed to create channel by non-uniform deposition during sputtering.

In this paper, nanotrenches will be made firstly by means of e-beam lithography and photolithography together with quartz and silicon substrate. Although these methods show promising and useful ways, simple fabrication of nanochannel is still challenging and demands high efforts. Moreover, an electrical sensing of single DNA molecule with the nanofluidic channel is a new challenging task as well. Therefore, simple fabrication techniques are suggested for generating SiO_2 without nanolithography, which is relatively with ease controllable by using oxidation condition. The first nano scale trench had V-groove shape cross-section, and so electrodes could be deposited on both sidewalls of nanotrench by metal evaporation. The sealing of channel was done by covering thin polydimethiysiloxane (PDMS) polymer sheet, finally. On the other hand, we developed the other method to fabricate complete SiO_2 nanochannels having widths between 50 and 450 nm through a simple LOCOS technique [9].

II. FABRICATION

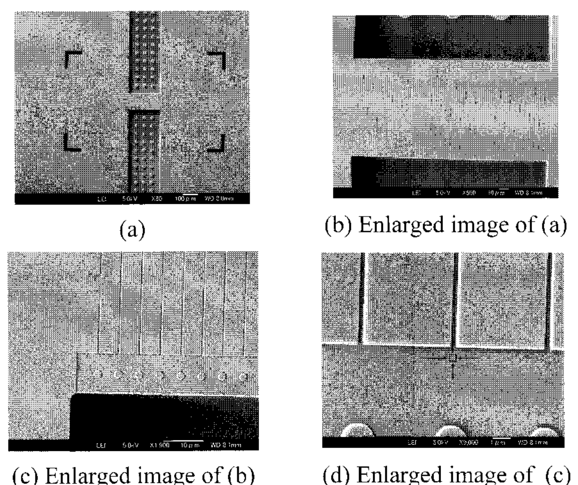
To fabricate nanochannels combined with micro scale structures, such as microchamber for sample flowing, hybrid pattern on one chip is necessary. Moreover, the material properties of biochip are also important. Therefore, in this paper, the following goals are targeted and in detail, various fabrication tools are investigated to find the proper condition of DNA manipulation. The goals: i) nanochannel structure fabrication on Si or Quartz substrate to be used as a master for replica of disposable polymer biochip fabrication later, ii) direct nanochannel structure fabrication with simple, low-cost way.

2.1 Fabrication of Nanotrenches on Silicon/quartz Substrate using E-beam Lithography

The selection in materials and composition of nanochannel is getting wider and more diverse as the importance and application increases. The underlying consideration of nanochannel in common is the combine of micro- and nano-structure, in other words, hybrid pattern. The necessity of inlet or outlet for target sample transportation invites the micro to millimeter scale structure. The flexible and proper co-existence of nano- and micro-, even millimeter layout must be considered as well as the related fabrication method.

The availability of material used for channel is quite restricted in pursuit with bio-application. The typical materials obtainable at our times are silicon, fused silica, fused quartz and polymer, etc.

The fabrication of nanochannels is preceded with target geometry of 100 nm x 100 nm in cross sectional area, made with silicon and fused quartz, respectively. The e-beam lithography produces nanotrench pattern and the other structures including microtrenches and inlet/outlet are constructed by conventional UV-photolithography. Even though quartz have an excellent optical and chemical property with good compatibility of bio-application, the fabrication of nanochannel is not simple than other material because of etch mask selection and the bonding process. High-resistant etching masks made of metal or silicon nitride are commonly required to fabricate channels on quartz substrates. We chose chromium metal layer as an etching mask since it can sustain under CF_4 and O_2 gas through RIE etching. If silicon nitride was selected as an etching mask, dry etching is not applicable for its weak resistance. In fabricating nanotrench with quartz, it is crucial process to make chromium mask pattern on quartz substrate by wet etching, because controlling of trench width is not a easy work and not repeatable.



(a) (b) Enlarged image of (a)

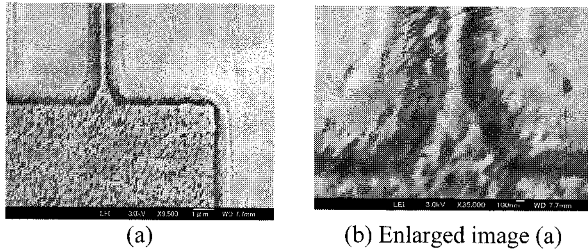
(c) Enlarged image of (b) (d) Enlarged image of (c)

Fig. 1. Fabricated nanotrench images based on silicon substrate.

Fig. 1 and fig. 2 show the realized nanotrenches with microchamber on Si and quartz substrate, where the size of channel width and depth is 100 nm, respectively.

The fabrication of nanochannel devices is completed after the open nanotrenched structure is sealed by PDMS sheet. The preparation of PDMS sheet is quite convenient to handle and repeatable process. Material is relatively cheap and easily available, what is better, bio- and chemically compatible and safe.

While the sagging of PDMS may cause the intrusion of channel, which causes sometimes troubles, resulting in failure and blocking the molecules flowing in channel. To prevent the undesirable obstacles of blocking channel from sagging, supporting pole was designed in microchannel part, which is shown in Fig. 1.



(a) (b) Enlarged image (a)

Fig. 2. Fabricated nanotrench images based on quartz substrate. (For SEM, thin chromium layer was deposited)

2.2 Fabrication of Silicon Dioxide Nanochannel without Nanolithography

We propose two different ways to form silicon dioxide nanochannels without nanolithography methods. One is to grow the thermal dioxide on the silicon trench structures, and the other is to reduce the silicon line structures using thermal oxidation. The first fabrication technique is described in Fig. 3(A). The micro-scale channel was patterned on the pre-cleaned silicon substrate by photolithography. Then, silicon was etched with deep RIE etching in 5 μm thick. Thermal wet oxidation was carried out at 1100 °C for 5 hrs, resulted in 1000 nm-thick silicon dioxide layer. The silicon dioxide layer can be generated under the condition of water vapor and high temperature. In the final obtained SiO_2 layer (Xox), the oxidized Si (consumed, Xs) is proportional to the obtained SiO_2 including the expanded dioxide layer, as follows.

$$Xs = 0.46\ Xox$$

To produce 1 μm-thick SiO_2 layer, 460 nm-thick of Si is converted and 540 nm thick of additional SiO_2 layer expanded. Therefore, silicon dioxide nanotrench can be fabricated by control of oxidation condition without using nanolithography described previously. So far, the silicon-based nanochannels have been widely used for its relatively easy handling in fabricating process. On the other hand, glass or quartz is complicate material for handling in micro processing although it is preferred for bio application devices for its superior property. The produced SiO_2 layer compensates both easy handling of fabrication and biocompatibility.

The second fabrication technique is based on the combination of anisotropic etching by potassium hydroxide (KOH) solution, local oxidation of silicon (LOCOS) and plasma etching of silicon as shown in Fig. 3(B). After Si_3N_4 layer is patterned by photolithography and RIE, silicon substrate is anisotropically etched by KOH solution (40 w%, 60°C) utilizing the relatively lower etching rate of <100> surface of single crystal silicon wafer. After LOCOS at 1100 °C, Si_3N_4 and Si are etched by deep RIE to form nanochannel. Finally, SiO_2 nanochannel is deposited by sputtering.

Fig. 4 expresses the silicon oxide layer generation before (a) and after oxidation (b), where microchannel trench on silicon substrate with cross-sectional area 1 μm X 3 μm became nanochannel trench with 100 nm x 3 μm

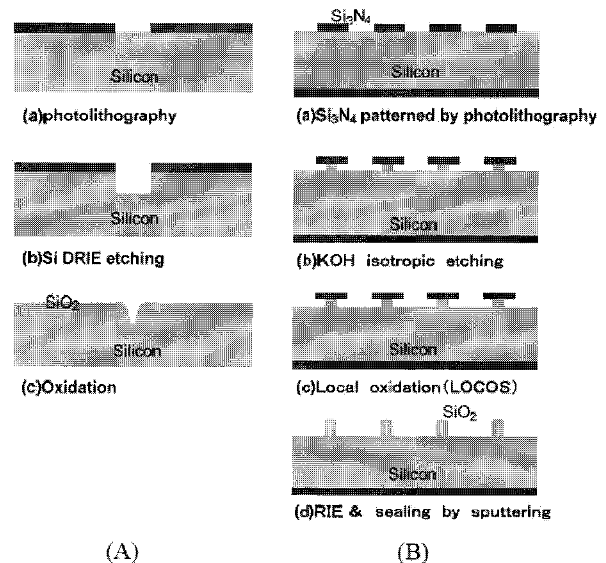
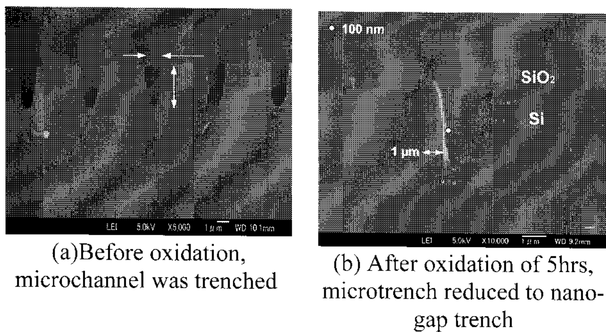


Fig. 3. Schematic views of fabrication process of silicon dioxid e nanochannel from trench structures (A) and line structures (B) on silicon substrate by growing dioxide.

cross-section area. Consequently the nanochannel geometry can be controlled by either oxidation process condition or adjust geometry of previous microchannel trench. Compared with other fabrication method, it has easy command of geometry control in nanochannel fabrication without nanolithography.

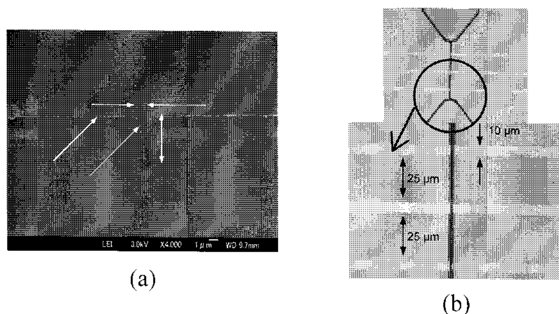
If we are utilizing a nanochannel as an electrical measurement platform for single DNA, silicon dioxide layer can works as an electrical isolation and passivation. Electrically isolated silicon dioxide compensates the hydrophobicity of PDMS and V-groove shaped channel secure proper space notwithstanding sagging of PDMS. Moreover, the cross-section of silicon dioxide nanochannel has a V-groove shape, which is very adequate for locating the metal electrode on both sidewalls of channel. Electrodes are placed on both sides of nanochannel and naturally disconnected, because the projectively exposed area decreases on V-groove part by non-uniform deposition of metal. The more inclined the wall is, the thinner the deposited metal layer is. Fig. 5 gives gold electrodes located on the side of nanochannel, depositing 100 nm gold on the adhesive 10 nm chromium layer.



(a)Before oxidation, microchannel was trenched

(b) After oxidation of 5hrs, microtrench reduced to nano-gap trench

Fig. 4. Cross sectional view of nanotrench with the 1 μm growth of SiO_2.



(a)

(b)

Fig. 5. Cross-sectional view of SiO_2 nanochannel with Cr/Au electrode on the sidewall (a) and optical microscopy images of nanochannel with 3 pairs of electrodes (b). (10 μm width electrode is patterned across the nanochannels and PDMS is covered on it.)

Fig. 6 shows the cross-sectional SEM images of nanochannels after nonuniform SiO_2 deposition by sputtering. It is also very attractive technique to fabricated nanochannel arrays without nanolithography.

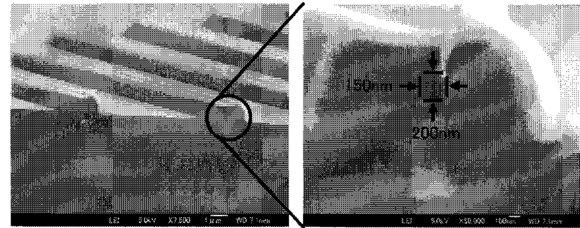
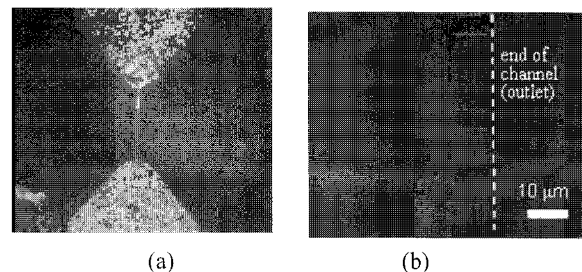


Fig. 6. Cross-sectional view of nanochannels after SiO_2 sealing.

III. DNA EXPERIMENT

As for the sealing of channel, PDMS was firstly chosen. Since the deposited metal electrode brings uneven surface on the top of a nanochannel, glass wafer bonding will not go with this case. In addition, the bowing of PDMS when covering the channel can be overcome because of enough space on the upper part of structure. Successfully fabricated nanochannel with three pairs of electrodes is shown in Fig. 5 by an optical microscopic image. Finally, we validated the DNA stretching successfully on silicon dioxide nanochannel prior to actual electrical measurement, using λ -phage DNA (48 kbp) stained with SYBR Gold as shown in Fig. 7. We used an epifluorescent microscope equipped with a 100 × oil immersion objective lens (N.A. 1.35) and a 100 W mercury arc lamp. Fig. 7(B) shows the stretched λ -phage DNA in nanochannels with a cross-section of 100 × 200 nm. The averaged stretched length is approximately 10 μm.



(a) (b)

Fig. 7. CCD image of λ-phage DNA molecule passing through nanochannel (A) and stretched single DNA (B) by capillary force.

If DNA is uniformly stained, the amount of emitted light is proportional to the size of the DNA molecule. The stretching state of DNA molecule is energetically favored when DNA molecules are transported into nanochannels having a cross-section comparable to the persistence length of the molecules. The comparison of stretching and behavior of DNA inside nanochannel will be more investigated with different substrate like silicon, quartz and silicon dioxide by passing through channels.

IV. CONCLUSIONS

Nanochannels fabrication with e-beam lithography on silicon and quartz substrates was carried out. Besides, we have presented new simple nanochannel fabrication methods without using nanolithography by generating silicon dioxide layer. The V-grooved shape of channel shows good adaptability for the metal electrodes to be located on the sidewall of nanochannel by thermal evaporation. In this paper, the nanochannels with different materials are introduced. Single DNA molecules stained with a fluorescent dye were transported into the nanochannels by capillary force and effectively stretched along these channels. Finally, various single DNA molecules will be sorted and observed by the diverse nanochannels.

ACKNOWLEDGMENTS

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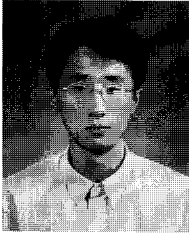

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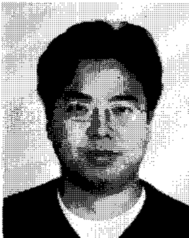

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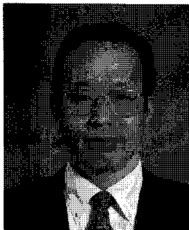

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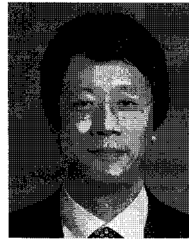

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