Polymer-based fluidic devices integrated with perforated micro- and nanopore membrane for study of ionic and DNA transport

Junseo Choi
Louisiana State University and Agricultural and Mechanical College

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POLYMER-BASED FLUIDIC DEVICES INTEGRATED WITH PERFORATED MICRO- AND NANOPORE MEMBRANE FOR STUDY OF IONIC AND DNA TRANSPORT

A Dissertation

Submitted to the Graduate Faculty of the Louisiana State University and Agricultural and Mechanical College in partial fulfillment of the requirements for the degree of Doctor of Philosophy

in

The Department of Mechanical Engineering

by

Junseo Choi
B.S., KyungHee University, 2004
M.S., KyungHee University, 2006
August 2013
To my parents,

Youhee Choi and Deoksoon Jeon,

and lovely family and friends
Acknowledgments

I would like to acknowledge the support and guidance provided by my major professor, Dr. Sunggook Park during the course of this study. I would like to thank the support and guidance provided by the members of my graduate committee, Dr. Michael C. Murphy, Dr. Dimitris E. Nikitopoulos, Dr. Ying Wang, Dr. William T. Monroe, and Dr. Robert Cook who is acting as the Dean’s representative.

Thanks to all members in Nanosystems Lab, especially to those who collaborated with me, Dr. Jeong Tae Ok, Dr. Bahador Farshchian, Dr. Alborz Amirsadeghi, Dr. Jiahao Wu, Lance Brumfield, and Steven M. Hurst. I also thank group members of the Microsystems Laboratory, Dr. Daniel Park, Dr. Byoung Hee You, Dr. Namwon Kim, Dr. Taehyun Park, Brooks Lowrey, and Chris Brown.
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Abstract

This study aims to develop a process, allowing a low-cost and high-throughput fabrication technique to produce freestanding polymer membranes having perforated micro- and nanopores, and also to design 3D micro/nanofluidic devices with the membrane, enabling a study of ions and DNA transport through nanopores.

Technically, high quality silicon stamps have designed and fabricated. Then, they have been used as molds for modified nanoimprint lithography that takes advantages of a sacrificial layer to obtain freestanding polymer membrane. This technique allows easy fabrication of large area, fully released polymer membranes containing perforated micro- and sub-micropores. The membrane with perforated micropores has been successfully integrated with microfluidic channels and used for in situ formation of lipid bilayer.

The membrane with nanopores (< 10 nm diameter) has been directly fabricated using modified nanoimprint lithography with a silicon microneedle stamp. Also, the pore size was reduced further (down to 10 nm) with a subsequent process such as pore reduction using polymer reflowing. Then, it was utilized for sensing and characterizing the ions and DNA transport through pores.
Chapter 1. Introduction

1.1 General background

Microfluidic devices have been attracting the attention of bioanalytical applications based on lab-on-a-chip technology. Advantages of lab-on-a-chip techniques include small sample and reagent consumption, accurate and sensitive analysis, and great portability [1]. These microfluidic systems have been formed with varied materials such as silicon, glass, quartz, and polymers. In the initial development, most of the microfluidic systems were realized in silicon or silica materials because of well-established fabrication protocols developed by the semiconductor industry. Although silicon and silica materials have excellent optical properties and high resistances to varied chemicals, their fabrication methods are complex in comparison with polymer materials. On the other hand, there are many methods to form microfluidic systems in polymers such as casting, embossing, injection molding, and imprinting which are mass-production, low-cost, and high-throughput methods for low-cost disposable devices [2]. Also, various polymers can be chosen to fabricate microfluidic systems with different mechanical, optical and electrical properties [2], and to modify their surface to attach various bioactive compounds [3].

As the field of lab-on-a-chip technology continues to grow thanks to its advantages, more sophisticated devices (i.e. three-dimensionally (3D) integrated micro- and nanochannel structures) require. Compared to 2D channels, the 3D structures allow achieving complex functionality and have thus been used in many microfluidic applications. Examples include patterning cells and proteins onto surfaces [4], controlling water-in-oil-in-water double emulsification [5], and mixing solutions in microchannels [6]. There are several strategies for fabricating 3D channel structures in polymers; for example, the “mortise and tenon” method [7,
8], the “membrane sandwich” method [4, 9, 10], the “solid-object printing” method [11], the “protruding mold feature” method [12], and the “perforated membrane” method [13, 14]. Among all of them, the integration of a perforated membrane with microfluidic networks has been extensively studied due to their various biological uses such as selective molecule separation, filtration and purification, biosensing, and single molecule analysis [15]. Particularly, a large surface-to-volume ratio of perforated membranes serves to facilitate fast solution exchange and molecule filtration based on their different sizes [16].

This research focuses on fabrication of polymer-based 3D micro- and nanofluidic devices integrated with perforated micro- and nanopore membranes and their use for single molecule (e.g. DNA, RNA, or proteins) analysis. Different methods have been developed to analyze single molecules such as atomic force microscopy (AFM), optical tweezers, and nanopore sensors. AFM and optical tweezers can provide mechanical and kinetic properties of individual single molecules, but cannot generate high-resolution sequence information [17]. On the other hands, nanopore sensors are based on electrical measurements of fluctuating ionic transport or conductance through nanometer-sized pores [18]. When the charged molecules pass through the nanopores by a gradient in concentration or voltage, the magnitude, duration, and frequency of the conductance variations can statistically be analyzed. This sensor promises to be a rapid, sensitive and label-free analysis technique. In early studies, protein nanopores such as α-hemolysin have been used as this sensor. Protein nanopores in aqueous solution have been implanted into a lipid bilayer formed across 30-100 µm diameter apertures to create working devices [19]. However, lipid bilayers are fragile and easily rupture and thus protein nanopores are not stable for an extended period of time.
Over protein nanopores, synthetic nanopores (e.g. solid state and polymer nanopores) have advantages such as increased stability over a wide range of temperatures and pHs, tunable pore geometry and surface chemistry, and the potential integration into lab-on-a-chip technologies. Typical technologies to produce perforated nanopores include anodization of aluminum [20, 21] and ion track etching of polymers [22-24]. However, these techniques cannot control pore arrangement or achieve narrow pore size distribution. Other technologies (e.g. a direct milling with a focused high-energy electron or ion beam and a shrinking of pre-patterned holes with either deposition of materials or exposure to a defocused beam of electrons, ions and photons) allow for fabrication of arbitrary nanopore patterns in the designated area on thin inorganic films (e.g. silicon nitride and silicon dioxide) [25]. However, these methods are slow and expensive. Compared to polymers, thin inorganic films to form nanopores are also not appropriate for disposable devices due to the high material and fabrication cost. A large number of identical samples are usually required to obtain reliable results in single molecule analysis.

Therefore, an in-depth effort to develop polymer-based 3D micro- and nanofluidic devices combined with micro- and nanopore membranes to be applied to bioanalytical fields is an imperative.

1.2 Goal and objectives of the project

The primary goal of this research is to develop a technology for low-cost and high-throughput fabrication of polymer-based micro- and nanopore sensors integrated with a freestanding polymer membrane having perforated micro- and nanopores for studying ions and DNA transport. The low-cost and high-throughput fabrication was achieved by the use of nanoimprint lithography (NIL).
The technical objectives to reach the goal include:

(1) design and fabricate high quality silicon or polymer stamps to be used as molds for NIL;
(2) develop the NIL process to achieve freestanding polymer membranes having micro- and nanopores;
(3) develop an integration process to assemble freestanding membranes into microchannels to build 3D micro- and nanofluidic devices; and
(4) apply the fabricated 3D micro- and nanofluidic devices to study the passage of ions and DNA through the pores.

1.3 Outline of the dissertation

This dissertation contains 8 chapters. The following is a brief overview of the contents of each chapter.

Chapter 2 provides a literature review regarding the background information on existing micro- and nanopore fabrication processes and various applications with micro- and nanopore devices. Also, ions and DNA transport in confined geometrics (e.g. nanochannel or nanopore) is another focus in this chapter. Chapter 3 discusses fabrication of freestanding polymer membranes having perforated pores down to sub-micrometer dimensions using a double layer resist and single step NIL. Chapter 4 discusses integration of the micropore membranes into microchannels and its application for the formation of lipid bilayers in the micropores. The studies in Chapter 3 and Chapter 4 serve as a foundation for Chapter 5 which discusses fabrication of freestanding polymer membranes having perforated conical-shaped nanopores down to sub-100 nm in diameter. Chapter 6 discusses voltage-driven ions and DNA transportation through the fabricated 3D nanopore devices. Chapter 7 discusses DNA uncoiling
and translocation through conical-shaped nanopores down to sub-10 nm by employing a pore-reduction process after initial formation of nanopores. Finally, a summary of the results and future work will be presented in Chapter 8.

1.4 References


Chapter 2. Research Background

This dissertation concerns the fabrication and applications of polymer membranes containing perforated micro- and nanopores and the microfluidic systems integrated with the membranes, which requires multidisciplinary backgrounds including fluid physics, surface and analytical chemistry, materials science and engineering, and micro- and nanofabrication technology. In this chapter, reviews of previous and current researches related to perforated micro- and nanopore membranes integrated with micro/nanofluidic systems and their applications are given.

This chapter is structured in the following way: (1) in the first section, brief information about membrane technology is described, (2) in the second section, biological applications of membrane integrated with microfluidic system are reviewed, (3) in the third section, the theory and models used to understand transport behavior of ions and charged molecules in confined geometries are discussed, and (4) in the fourth section, the fabrication methods to make micro- and nanopores are reviewed.

2.1 Introduction

Membranes play an important role not only in natural/biological systems but also in many industrial systems. An example of natural/biological membrane systems is cell membranes or plasma membranes. A cell membrane or plasma membrane consists of a lipid bilayer in which various and enormous proteins are embedded, separating the interior of all cells from the outside environment. Cell membranes play a significant role in many cell functions. In addition to protecting cells from outside forces, the cell membranes are involved in a various cellular processes such as the formation and structure of lipid micro-domains [2], peptide/lipid
interactions [4], cell-adhesion and cell signaling [6] by controlling the movement of substances in and out of the cells.

Similar to the cell membrane, a synthetic membrane can be defined as an interface between two adjacent phases acting as a selective barrier, regulating the transport of substances between the two compartments [8]. Unlike other conventional mass separation techniques (e.g. distillation, crystallization, solvent extraction), membrane separation is unique and has many advantages allowing it to be widely used. For example, separation can be performed continuously without using additives, it can be carried out isothermally at low temperature and at low energy consumption, and its up- and downscaling is relatively simple. Consequently, membranes are used on water and wastewater treatment, biopharmaceutical separations, gas separation, and fuel cells [16].

By combing with microfluidic techniques, the advantages mentioned above have been maximized due to some reasons such as small sample and reagent consumption, accurate and sensitive analysis, and great portability [17]. There are several methods to integrate membranes with microfluidic networks. According to a recent review by de Jong and co-workers [18], the different approaches of integrating membrane functionality on chip can be categorized into the following four types: (1) direct incorporation of membranes, (2) membrane preparation as part of the chip fabrication process, (3) in-situ preparation of membranes, and (4) use of membrane properties of bulk chip material.

Compared to an integration approach (3) in-situ preparation of membrane and (4) use of membrane properties of bulk chip material, the other two approaches can easily integrate a membrane into a microfluidic chip by clamping or gluing. Also, various materials can be chosen as membranes (by fabricated membrane or purchased commercial one) and additional chemical
treatments can be possible before integration. Micro- and nanopore applications integrated with microfluidic system as approach (1) and (2) focus therefore on in next section.

2.2 Micro- and nanopore technologies for bio-applications

Most applications of membranes in microfluidics are found in analytical chemistry. Since analytical equipment is often sensitive to sample composition, in most cases samples cannot be directly analyzed and require a pre-treatment. This may include selective removal of large components, impurities and dust on one side and low molecular weight components such as salts on the other. Moreover, often the concentration of the components of interest is below the detection limit of the analysis equipment. For these reasons, membrane operations are essential in biological and medical applications. In the following section, examples will be given of biological and medical applications of membranes in microfluidics. The aim is to show various applications of membranes in microfluidics without discussion in much detail.

2.2.1 Separation and sorting of biomolecules

Separation and sorting is used for isolation and purification of molecules from various biological feed streams. One example is the separation of red blood cells (RBCs), white blood cells (WBCs) and platelets from whole blood cellular components [19, 20]. Usually, isolation of those cells was based on the size-exclusion because it is a passive and simple technique that does not require complicated and expensive equipment. Besides blood separation, other cells of interest such as circulating tumor cells (CTCs), nucleated RBCs (NRBCs) and microorganisms are also present in peripheral blood, which are of clinical significance and important for disease diagnosis and fundamental research [21, 22]. Also, hematopoietic stem cell, one of the most
documented approaches in regenerative medicine and promise treatment for a multitude of diseases and disorders, was separated from bone marrow [23, 24].

2.2.2 Biosensing

In many technological areas including the pharmaceutical industry, medical diagnostics, and detection of hazardous biomolecules, biosensing is important. A typical example is a glucose sensor. Glucose is one of the most important molecules in living animals. When the pancreatic function is impaired (Type 1 diabetes) or the response by the body to insulin diminishes (Type 2 and gestational diabetes), the glucose concentration in blood fluctuates. Both lead to abnormally high blood sugar levels (hyperglycemia). Therefore, monitoring glucose is important for effective treatment of diabetes, becoming one of the most common and serious diseases especially in advanced nations nowadays. An autonomous implantable biosensor equipped with nanoporous membranes would enable long-term continuous monitoring of the glucose concentration, alleviating diabetic patients’ physical pain caused by daily blood-drawing for screening [25, 26].

2.2.3 Single molecular analysis

Another type of biosensor with porous material is used for single molecule analysis. There are different methods to analyze single molecules such as atomic force microscopy (AFM), optical tweezers, and nanopore based sensors [27]. AFM and optical tweezers can give mechanical and kinetic information of individual single molecules, but cannot generate high-resolution sequence information. From this point of view, nanopore (e.g. biological or synthetic nanopore) based sensing is attractive in terms of DNA sequencing because it is a label-free,
amplification-free, single-molecule approach that can be scaled for high-throughput DNA analysis. Moreover, it typically requires low reagent volumes, benefits from relatively low cost

Table 2.1 Different sensing modalities for nanopore sensors and the challenges they face.

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<th>Description of technique</th>
<th>Potential challenges</th>
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<td>Hybridization-assisted nanopore sequencing</td>
<td>High spatial resolution required; complex algorithms needed for analysis.</td>
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<td>Sequencing by exonuclease digestion</td>
<td>Requires sequential passage of mononucleotides in the order in which they are cleaved [1].</td>
</tr>
<tr>
<td></td>
<td>Sequencing by synthesis</td>
<td>Retaining processing enzymes (DNA polymerase) at the pore; achieving long read lengths and maintaining enzyme activity under a voltage load [3].</td>
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<tr>
<td></td>
<td>Duplex interrupted DNA sequencing</td>
<td>Converting large genomic ssDNA fragments to duplex interrupted structure [5].</td>
</tr>
<tr>
<td><strong>Optical readout</strong></td>
<td>Optical recognition of converted DNA</td>
<td>Complex and error-prone DNA conversion steps; high density, &lt;2-nm-diameter nanopore arrays needed [7].</td>
</tr>
<tr>
<td><strong>Transverse electron tunnelling</strong></td>
<td>Tunnelling detector on a nanopore (metal, graphene, carbon nanotubes)</td>
<td>Precisely controlling orientation and position of nucleotides in the gap; slow translocation rates required to sufficiently sample over noise; nucleotide dependent tunnelling currents need to be measured in solution [9-13].</td>
</tr>
<tr>
<td><strong>Capacitive sensing</strong></td>
<td>Metal-oxide-semiconductor nanopore capacitor</td>
<td>Must operate in high-ionic-strength solution with negligible drift and leakage; DNA translocation rates need to be substantially reduced [14, 15].</td>
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and supports long read lengths. In the field, the major challenges are to reduce the speed of DNA translocation and to improve the sensitivity of the approach. Table 2.1 shows different sensing modalities for nanopore sensors and the challenges they face.

2.2.4 Immunoisolation

Protecting implanted cells or drug release systems from an immune reaction is known as immunoisolation. It is usually carried out by encapsulation using a nanoporous semipermeable membrane. The use of nanoporous membranes serves to isolate the transplanted cells from the body’s immune system. These pores are large enough to allow small molecules such as oxygen, glucose, and insulin to pass, but are small enough to impede the passage of much larger immune system molecules such as immunoglobulin. For example, in the artificial pancreas, insulin secreting pancreatic cells are encapsulated inside an immunoisolating device and a semipermeable membrane acts as an interface between the cells and the body. Desai et al. have explored using nanoporous silicon interfaces prepared by microfabrication techniques in implantable artificial pancreas to treat diabetes [28-30].

2.3 Transportation in confined geometries

As mentioned in the above section, there are several applications of micro- and nanopore techniques in biological fields. Especially, this research focuses on the fabrication of perforated micro- and nanopore membranes integrated with micro- and nanofluidic systems and its use for single molecule (e.g. DNA, RNA, or proteins) analysis. Therefore, the theory and models used to describe transport in confined geometries should be addressed.
2.3.1 Ion transportation in confined geometries

Electrokinetic transport produced from both electrophoretic flow\(^1\) and electroosmotic flow\(^2\) is usually used as the transport mechanism in confined geometries (e.g. nanochannel or nanopore) [31]. Although hydrostatic transport, another method of transporting analytes by pressure induced flow [32], has no dependence on solution chemistry and analyte charge, a detrimental effect of this technique is the parabolic velocity profile across the channel width and depth, resulting in a broad range of analyte speeds. Also, pressure driven flow results in an average velocity that scales with square of the channel diameter, making it unfavorable for

---

\(^1\) Electrophoretic flow is the motion of charged colloidal particles or molecules through a solution influenced by an applied electric field.

\(^2\) Electroosmotic flow is the motion of liquid induced by an applied electric field.
confined geometries [33]. The electrokinetic transport in nanoconfined systems differs from that in microfluidic systems because the influence of the electrical double layer (EDL) is more prominent. According to the Gouy-Chapman-Stern model, the EDL is composed of the inner Helmholtz plane, the outer Helmholtz plane, and a diffusion layer, as shown in Figure 2.1 [31]. The inner Helmholtz plane layer ($\psi_i$) consists of nonhydrated co-ions and counterions, whereas the outer Helmholtz plane layer ($\psi_d$) is built up of only hydrated counterions. The diffuse layer is defined beyond the outer Helmholtz plane. At the slip plane, the $\zeta$ potential can be experimentally investigated, and as the distance between the outer Helmholtz plane and the slip plane is negligible in most cases, the $\zeta$ potential is usually equal to $\psi_d$. The potential distribution in EDL is expressed by the Poisson-Boltzmann equation [31],

$$\nabla^2 \psi = \frac{d^2 \psi}{dz^2} = -\frac{e}{\varepsilon_0 \varepsilon_r} \sum_{i} n_i^{\infty} z_i \exp\left(-\frac{z_i e \psi(z)}{k_B T}\right)$$  \hspace{1cm} (2.1)

where $\psi$ is the electric potential due to surface charge density ($\sigma_s$), $e$ is the charge of the electron, $\varepsilon_0$ is the permittivity of free space, $\varepsilon_r$ is the relative permittivity, $n_i^{\infty}$ the is bulk volume density, $z_i$ is the valency of ion, $k_B$ is the Boltzmann constant, $T$ is the absolute temperature. The Poisson-Boltzmann equation is a second-order elliptic partial differential equation, and can be solved analytically by assuming that the surface potential is small everywhere in the EDL and by expanding the exponential, which leads to the Debye-Huckel approximation [31],

$$\nabla^2 \psi = \frac{d^2 \psi}{dz^2} = \kappa^2 \psi(z)$$  \hspace{1cm} (2.2)

where
\[ \kappa = \left( \frac{e^2 \sum_l n_l^0 z_l^2}{\varepsilon_0 \varepsilon_r k_B T} \right)^{1/2} \]  

(2.3)

\( \kappa \) is called the Debye-Huckel parameter and is mainly dependent on the bulk volume density \( n_l^0 \). The potential decays exponentially in the diffuse layer with the characteristic distance given by the Debye length \( \lambda_D = \kappa^{-1} \). This value corresponds to the thickness of the EDL. For a symmetrical \( z_l:z_l \) electrolyte with concentration \( c_l \) at 25 °C, the value of the Debye length \( \lambda_D \) (unit is meters) can be written as

\[ \lambda_D = \frac{3.04 \times 10^{-10}}{z_l \sqrt{c_l}} = \frac{2.15 \times 10^{-10}}{\sqrt{I_s}} \]  

(2.4)

where the ionic strength \( I_s \) is

\[ I_s = \frac{1}{2} \sum c_l z_l^2 \]  

(2.5)

For \( I_s = 10^{-2} \, \text{M} \), \( \lambda_D = 3.04 \, \text{nm} \), while for \( I_s = 10^{-4} \, \text{M} \), \( \lambda_D = 30.4 \, \text{nm} \) [34].

The Debye length typically ranges from 1 to 100 nm in aqueous solutions depending on the bulk ion concentration. At high bulk ion concentrations, at which the Debye length, \( \lambda_D \), is much smaller than a typical nanochannel size of tens or hundreds of nanometers, \( h \), the ion concentration in the nanochannel is the same as that outside in the reservoirs. The electric potential remains zero in most parts of the channel. On the contrary, at low bulk ion concentration, the electric potential inside the nanochannel is altered by the overlap of two EDLs from opposite walls. Also, the counterions are accumulated while co-ions are excluded from the channel resulting in a unipolar solution in the nanochannel. The reason is that the EDL will
overlap in the nanochannel and affect electroosmosis, which is no longer plug flow but instead follows the electric potential, \( \psi(z) \). Under low ionic strength conditions, the electroosmosis velocity \( u_{eo} \) is dependent on the potential distribution between two parallel plates and is given by [31]

\[
    u_{eo} = -\frac{\varepsilon_0 \varepsilon_r \zeta E_x}{\eta} \left( 1 - \frac{\psi(z)}{\zeta} \right)
\]  

(2.6)

where \( E_x \) is the electric field in the \( x \) direction and \( \eta \) is the dynamic viscosity of the fluid. Meanwhile, the average concentration is determined by the surface charge density \( (\sigma_s) \),

\[
    \sigma_s = \sum_i \frac{q_i}{A}
\]  

(2.7)

where \( q_i \) is the net charge of the ion and is given by \( q_i = z_i e \) and \( A \) is the surface area. This indicates that the electrostatic effect arising from the surface charge can influence throughout the channels having the size of about the Debye length. If the surface is negatively charged, the nanochannel will be rich in cations. On the contrary, positively charged surface will yield the accumulation of anions inside the channel. Such effect is not observed in microfluidic channels because of its smaller surface-to-volume ratio. Consequently, the effect of surface charge on the ionic composition and transport in nanochannels leads to various unique phenomena that cannot be achieved in microfluidic channels.

Ion transport is usually assessed by the Nernst-Planck equation [35],

\[
    J_i = D_i \nabla c_i - \frac{z_i F}{RT} D_i c_i \nabla \Phi \pm v_c c_i
\]  

(2.8)
where $J$ is the flux per area, $D_i$ is the diffusion coefficient of ion $i$, $F$ is the Faraday constant, $R$ is the gas constant, $\Phi$ is the electric potential, and $v_c$ is the convective velocity filed. Equation 2.8 is the sum of three terms, as follows: (1) Fick’s first law, which specifies the diffusion of molecules due to a chemical potential gradient (concentration gradient), (2) the flux due to the electric potential $\Phi$, and (3) convective transport, which can be due to pressure, mechanical stirring, and external fields such as electric and magnetic fields.

**2.3.2 Biomolecules transportation in confined geometries**

Biomolecule transport can also be modeled using the Nernst-Planck equation [35]. However, biomolecules are affected by the molecule size with respect to the lateral channel dimensions, and also by considerations of molecular entropy. Therefore, it is essential to understand free DNA behaviors in advance. The conformation of DNA in solution is decided by

![Figure 2.2](image)  
Figure 2.2 Three different regimes in polymer theory of confined DNA. (Top) In free solution, DNA can form a coil with a characteristic length scale $R_G$. (Center) In the de Gennes regime, the DNA can still coil up locally and is usually modeled as a series of non-interacting blobs. (Bottom) In the Odijk regime, the DNA is extended as series of undulations, with a characteristic length $\lambda = (PD^2)^{1/3}$, between the channel walls.
a balance between entropic effects and excluded volume effects\(^3\). The entropic effects work to minimize the volume of the DNA coil and the excluded volume effects work between interacting DNA segments which cause their expansion. This controversy provides an rms end-to-end length (represents Flory radius, \(R_F\)) of the free DNA coil [36],

\[
R_F \sim (w_{\text{eff}} 2P)^{\frac{1}{5}} L^{\frac{3}{5}}
\]  

(2.9)

where \(w_{\text{eff}}\) is the effective width, \(P\) is the persistence length (the bending stiffness of the polymer) and \(L\) is the contour length of the DNA (the number of monomers in the chain \(\times\) the distance per monomer) [37].

The DNA confined to spaces of dimensions smaller than the radius of gyration, \(R_G\), is based on the theory of de Gennes and Odijk and it can be separated into two regimes. The boundary between the two regimes is the persistence length. DNA confined in channels with a cross-section much larger than \(P\) can fold back on itself and coil up, dominating exclude-volume effects. In this regime, the DNA can be modeled as a series of non-interacting blobs. In each blob, the DNA acts as it would in free solution. This gives to an extension, \(r\), of the DNA along the channel [36],

\[
\frac{r}{L} \propto \left( \frac{w_{\text{eff}} P}{D^2_{av}} \right)^{\frac{1}{3}}
\]

(2.10)

where \(D_{av} = \sqrt{D_1 D_2}\) is the geometric average of the channel width, \(D_1\), and height, \(D_2\), of a rectangular channel.

\(^3\) Large flexible coil or globular polymeric solutes occupy volume and reduce the amount of space available in the solution, raising the effective concentration of physically large species.
For smaller channel diameters less than the persistence length, the energy to form a loop is greater than the thermal energy, $k_B T$, decreasing the probability of loop formation. In this case, excluded-volume effects are not dominant and the end-to-end distance follows the theory of Odijk. It was based on the average projection of the characteristic deflection length, $\lambda$ (see Figure 2.2),

$$\frac{r}{L} \sim 1 - A \left(\frac{D}{P}\right)^{2/3}$$  \hspace{1cm} (2.11)

or for a rectangular cross section,

$$\frac{r}{L} \sim 1 - B \left[\left(\frac{D_1}{P}\right)^{2/3} + \left(\frac{D_2}{P}\right)^{2/3}\right]$$  \hspace{1cm} (2.12)

Here, $A$ and $B$ are $\sim 0.17$ and $\sim 0.091$, respectively.

### 2.4 Fabrication of micro- and nanopores

Porous materials are often distinguished based on pore size, size distribution, shape, and order. Porous materials are considered uniform if the size distribution is narrow as opposed to a wide pore size distribution. The pores can be cylindrical, conical, slit-like, or irregular in shape. They can be well ordered with a vertical alignment as opposed to a random network of tortuous pores [38]. Most widely used porous structures are random nanopores produced in organic and inorganic membranes through chemical synthesis, which have been used in separation and catalytic applications. For the analysis of biomolecules such as DNAs, however, it is preferred to use geometrically well-ordered pores over the random pores. Therefore, the literature survey on the fabrication of micro/nanopores in this section will focus only on methods to produce
geometrically well-ordered micro/nanoporous structures. The review will be organized according to the classification of material types.

2.4.1 Fabrication on inorganic materials

*Aluminum anodization* Anodic aluminum oxide films formed by the electrochemical oxidation of aluminum have been extensively utilized. Initial porous alumina membranes have been formed with honeycomb-like pore structure, having short distance ordering [39, 40]. The pore formation mechanism is followed by four steps: (1) at the beginning of the anodization, the barrier film (consists of non-conductive oxide) covers the entire surface of the aluminum, (2) the electric field is focused locally on fluctuations of the surface, (3) this leads to field-enhanced or/and temperature-enhanced dissolution in the formed oxide and thus to the growth of pores, (4) since some pores begin to stop growing due to competition among the pores, the current decreases again. In this stage, pores grow in a stable manner.

The pore geometry and morphology can be controlled by the conditions during the anodization processes. In recent years, great success has been achieved in the preparation of AAO membranes with highly ordered nanochannel arrays. Conventional anodizing of aluminum could produce an ordered nanochannel array with a limited range of pore sizes through a self-organized process [40]. The degree of the self-organization in the hole-array depends on the anode voltage as well as the acid used in fabrication. However, the most effective method for developing ordered porous anodic alumina with large dimension is the pretexture process, in which a shallow ordered pattern is first produced on the surface of an aluminum wafer, and the ordered channel structures are obtained by the following anodization process. Using this method, Masuda et al. produced porous anodic alumina with almost defect-free, ordered channel arrays.
The size of the membrane was on the order of millimeters, with a channel density of $10^{10}$ cm$^{-2}$ and an aspect ratio over 150. The smallest channel diameter was about 70 nm with 100 nm intervals. The anodization of aluminum was conducted in oxalic acid. Further investigation has indicated that the channel interval can be modified by controlling the pretextured pattern interval or the applied voltage [42]. Recently, Lee et al. have used a hard anodization process that enables fast fabrication of AAO membranes with pore sizes down to 40 nm making them ideal for size sorting a wide range of molecular sizes [43].

**Micromachining (high end fabrication tools)** Recently, micro- and nanofabrication technology has emerged as an attractive way to make ordered and cylindrical arrays of micro- and nanopores on silicon and silicon nitride surfaces [25, 28-30]. Considerable effort has been devoted to developing porous silicon and silicon nitride membranes using lithography in order to overcome the problems such as broad pore size distributions, poor mechanical properties, and biochemical instability associated with the conventional polymeric membranes. For example, Tong et al. have fabricated an array of very uniform cylindrical nanopores with a pore diameter as small as 25 nm in an ultrathin micromachined silicon nitride membrane using FIB etching [44]. Desai et al. proposed micromachined nanoporous biocapsules with well-controlled pore sizes as small as 7 nm to provide immunoisolating microenvironments for encapsulated cells using sacrificial layer etching [45].

Meanwhile, Li and co-workers reported a novel technique, ion beam sculpting, by which they fabricated single nanopores in Si$_3$N$_4$ membranes with nanometre control and used it for DNA sensing [46]. They used a dedicated ion beam machine that uses a focused ion beam to mill a tiny hole in the membrane. Feedback from ion detectors below the membrane provided signals
to indicate when to stop the milling. Interestingly, they observed that, depending on the ion beam current and temperature, pores could enlarge and shrink, allowing the fine-tuning of pores in the nanometer range. Storm and co-workers discovered that high-intensity wide-field illumination with electrons slowly reduced 20 nm pore up to sub 10 nm [47]. Large holes, with a diameter greater than the membrane thickness, grew in size, whereas small holes shrunk. It has also been shown that a pore can be directly drilled in a membrane by a locally focused electron beam in a TEM [48, 49], making laborious preparatory electron-beam lithography of larger nanopores unnecessary. Again, such pores can be modified with the wide-field TEM illumination. These processes have good control of pore diameter and are easy to integrate into microfluidic devices. However, the production of these membranes is still costly.

2.4.2 Fabrication on organic materials

**Ion-track etching** This technology can be applied directly to most polymers and wide variety of materials, including metals [50]. When dielectric materials, such as polymers, ceramics and minerals, are bombarded by swift ions, latent tracks are formed along the path of the ions [51]. Ion track materials can be divided into two categories: (a) single-tracked and (b) multiple-tracked materials. Single-tracked materials can be produced by controlling the beam optics and the frequency of ions passing. Commonly tracked materials are Makrofol-KG, Kapton-H, PVDF, mica films, cellulose nitrate, CR-39 and Lexan polycarbonate. Swift ion beams are produced by cyclotrons or linear accelerator and the radiation is characterized by extremely high linear energy transfer [50]. The conventional ionization radiation sources, such as radioactive isotopes or electron accelerators, are less sophisticated and less expensive and are mostly used in the industrial processes [50]. After irradiation, the materials are subject to chemical etching that
preferentially removes the latent ion track. This etching process results in pore formation in the material. Etching is the pore-size-determining and pore-shape-determining stage of the technology. In a homogeneous isotropic medium, mainly two influential parameters describe the etch process: the bulk etch rate $V_B$ and the track etch rate $V_T$ [50]. The ratio of track etch rate to the bulk etch rate is called the track-etch-ratio. When $V_T$ is larger than $V_B$, pores turn out to be cylindrical as opposed to conical. In other words, high track-etch-ratio yields cylindrical pores, where as low track-etch-ratio yields conical pores. The arctangent of the inverse track-etch-ratio ($V_B/V_T$) yields the half cone angle of the pore. The bulk etch rate depends on the material, on the etchant composition and on the temperature [51]. The track etch rate depends on the sensitivity of the material, irradiation conditions, post-irradiation conditions and etching conditions [50, 51].

**Nanoimprint lithography (NIL)** is a replication technique that provides a resolution unmatched by many other techniques, while at the same time offering parallel and fast fabrication of micro and nanostructures. This enables a step into fields where large areas are covered by nanostructures, or a number of identical structures for statistical evaluation are needed [52]. Moreover, as imprint lithography is not based on chemical modification of resist structure by radiation, the resolution is not limited by factors such as wave diffraction, scattering and interference. However, there are major challengers such as to reduce stress and deformation of molded polymers during demolding, to improve anti-stick coatings on a mold, and to fabricate reliable stamps with sub 100 nm features [34, 52].

NIL was first reported as molding into thermoplastic polymer resists, and is therefore often referred to as hot embossing lithography (HEL) [53-55]. The unique advantage of a thermoplastic material is that the viscosity can be changed to a large extent by simply varying the
temperature. The NIL process starts with heating a polymer substrate above its glass transition temperature \( T_g \). During this step, the thermoplastic film is compressed between the stamp and substrate and the viscous polymer is forced to flow into the cavities of the mold, conforming exactly to the surface relief of the stamp. When the cavities of the stamps are filled, the polymer then is cooled down, while the pressure is maintained. After relieving the pressure, the stamp can be retrieved (demolded) without damage, and reused for the next molding cycle.

There have been efforts to use NIL in fabricating membranes with perforated micro- and nanopores. Heyderman and co-workers used it to have a high volume fabrication process for nano scale perforated membranes in Si\(_3\)N\(_4\) substrate [56]. NIL process has been employed to imprint in a PMMA resist layer spin coated over a Cr coated double sided silicon nitride wafer, defining porous structures with dimensions as small as 140 nm in diameter. The nitride wafer was preferentially etched from the backside using RIE and wet chemical etching using KOH, which resulted in a Si3N4 membrane with perforated nanopores. Schift and co-workers reported fabrication of 3 \( \mu \)m diameter holes in a 1 \( \mu \)m thick polystyrene layer [57]. In order to produce a standing structure, a Lift off resist (LOR) was spin coated before the resist coating. After NIL and an O\(_2\) plasma etching to open windows down to the LOR layer, The LOR was partially dissolved to have a polystyrene membrane with perforated micropores which were supported by LOR bridges.

Despite such demonstration of using NIL for porous membranes, no effort has been made for developing technologies to fabricate fully-released polymer membranes with nanopores and micro/nanofluidic devices integrated with the porous membranes in a modular manner. Such technologies are critical to the development of low cost bioanalytic micro/nanofluidic devices and systems, which are the goal of this dissertation.
2.5 Conclusions

Micro- and nanopore membranes combined with microfluidic techniques offers great potential for biological applications. Among them, membranes with perforated nanopores have received great attention as low cost and fast DNA sequencing tools overcoming an existing method of sequencing the human genome which is too slow and expensive [58]. Several methods have been used to fabricate single nanopore such as anodizing of aluminum, micromachining of insulating materials, and ion track etching of polymers but these methods are not suitable for mass production of nanopores with defined pore dimensions. Therefore, high throughput and inexpensive technique to fabricate perforated nanopore with sub 10 nm size should be developed. In this point of view, NIL is expected to be the most suitable method.

2.6 References


Chapter 3. Low Cost Fabrication of Micro- and Sub-micropores in Freestanding Polymer Membranes

3.1 Introduction

The ability to imitate the architecture of natural systems is of great interest because it provides a platform for various fundamental and applied research [1]. One of the various mimic structures is a membrane with perforated pores. It plays an important role in various applications. Particularly, membrane technologies have been widely used in modeling cell membranes to study specific biological phenomena occurring at the cell membranes such as the formation and structure of lipid micro-domains [2], peptide/lipid interactions [3], and cell-adhesions [4]. Also, membranes have been used as a filter to separate particles and biomolecules in microfluidic system [5-7], a shadow mask for surface patterning [8-10], and a components in three dimensional microfluidic devices [11-13].

Membrane structures with perforated micro- and nanopores have been produced by a number of methods. Commercially available membranes are fabricated by ion track etching in polycarbonate or anodization of aluminum, which produces randomly distributed or a hexagonal array of nanopores, respectively, with pore diameter as small as 10 nm. Meanwhile, perforated micro- and nanopores in specific locations in a membrane with their size controlled have been fabricated owing to developing micro- (e.g. direct photolithography [14], reactive ion etching [15], phase separation [16, 17], and soft lithography [5, 8, 9]) and nanofabrication techniques (e.g. focused ion beam and electron beam lithography [18-21]). However, those methods are slow and expensive, and thus are not able to provide many identical samples, which are often required for study of many biological events.
This chapter shows a simple and high throughput fabrication process to produce fully released polymer membranes with controlled pore size and location that can be easily integrated into a microfluidic system in a modular approach. A modified nanoimprint lithography (NIL) process was combined with a sacrificial layer technique. Although Schift et al. [22] produced perforated membrane structures in thin polystyrene through a similar method, their membrane was sustained by polymeric columns due to low mechanical stability. The self-supporting mechanical stability of the released membrane, as large as 10.16 cm in diameter by using UV

Figure 3.1  AutoCAD design for the photomasks in order to fabricate silicon stamps to be used for small area imprinting (a) and large area imprinting (b). Scale bar: 50 µm.
curable polymer resist, SU-8 was achieved. Perforated pores as small as 500 nm diameter were demonstrated.

3.2 Experiments

3.2.1 Photomask Design

Photomasks were used for patterning positive photoresist coated on silicon wafers. Two photomasks were designed and used. The design of the first photomask is shown in Figure 3.1(a). The structures on the photomask were comprised of dots and linear gratings of varying diameters, widths and periodicities. It was used for small area patterning (e.g. 2 cm × 2 cm). The other photomask was designed for large area patterning. The structures on the second photomask consist of linear gratings with 5 µm width and 15 µm period covered over the entire area of 10.16 cm in diameter. It was shown in Figure 3.1(b). These photomasks with a Cr layer as a clear or dark field pattern were custom ordered from Advanced Reproductions (North Andover, MA).

3.2.2 Silicon stamp fabrication

Silicon stamps with micropillar structures were fabricated using a combination of photolithography and micromachining techniques, as shown in Figure 3.2(a). (100)-oriented silicon substrates coated with a 100 nm thick chromium (Cr) layer were used as a substrate. After oxygen plasma treatment and dehydration at 110 ºC, the silicon substrate was spin-coated with a 1.3 µm thick layer of a positive photoresist S1813 (Shipley Co.) at 3500 rpm for 45 s. This is followed by a pre-exposure bake at 115 ºC for 60 s. Photolithography was done with custom designed photomasks (Advance Reproductions Co.) in a UV exposure station (UL7000-OBS Aligner and DUV exposure, Quintel Co.) in a class 100 cleanroom in Center for Advanced
Microstructures and Devices (CAMD). Especially, it was carried out two times after 90° rotating the photomask having line gratings in order to pattern dot structures.

The exposed wafer was then developed in MF319 developer solution (Rohm and Haas Co.) for 90 s, followed by washing with deionized water for 5 min and drying with N₂ gas. Any residual layer in the recessed area of resist patterns was removed by etching with oxygen plasma for about 10 s. Then, the exposed Cr was wet-etched in order to open the window. The pattern transfer on the silicon substrate was achieved using a reactive ion etching (RIE) and deep reactive ion etching (DRIE) process in order to obtain low and high aspect ratio structures, respectively. Prior to using the stamps for NIL, the stamp surfaces were treated with a fluorinated silane, (heptadecafluoro-1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11-undecfluoro-1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,12,12,12-trichlorosilane (Gelest Inc.), in the vapor phase to reduce adhesion to the resist.

3.2.3 SU-8 membrane fabrication

For the fabrication of SU-8 membranes, NIL was combined with a sacrificial layer technique. The process is shown in Figure 3.2(b). A 10.16 cm quartz wafer was spin-coated with a double resist layer. A 1 µm thick LOR layer (LOR-7B, MicroChem Co.) was first spin-coated at 1500 rpm for 60 s as a sacrificial layer, then baked at 150 °C for 5 min. On the LOR layer, an SU-8 (SU-8.2 or SU-8.5, MicroChem Co.) layer was spin-coated, followed by a two-step baking process at 65 °C for 5 min and 95 °C for 5 min, respectively. The thickness of the SU-8 layer was controlled to be similar to or lower than the height of the pillars in the stamp used. For example, when imprinting with a stamp with pillars of 3 µm diameter and 3.7 µm height, the SU-8 layer thickness was ~3.7 µm. NIL was then performed in the resist layer with a commercial nanoimprinter (Eitre® 6, Obducat), which allows for both thermal and UV imprinting. The
nanoimprinter is equipped with a high-power, flash-type UV lamp (maximum power, ~1.3 W) with 250-400 nm wavelengths for UV curing. Details on imprinting process conditions will be described later.

After imprinting, the thickness of the residual SU-8 layer was in the range of 0-200 nm. In order to ensure the perforation of the pores through the SU-8 layer, the sample was treated with an oxygen plasma at the power and gas pressure of respective 150 watt and 400 mTorr for 1 min. The membrane with perforated micropores was finally released by dissolving the sacrificial LOR layer with a MF319 solution (Rohm and Haas Co.). The thickness of the released membrane was similar to the coating thickness.
3.3 Results and discussion

3.3.1 Low aspect ratio silicon stamp fabrication

In order to transfer patterns to the underlying silicon after photolithography into a photoresist layer, a dry silicon etch process was performed with a fluoride gas plasma in inductively coupled plasma (ICP) etch equipment (PlasmaLab system 100/ICP180 without cryogenic cooling system, Oxford Instruments Co.) at CAMD. Mixed fluoride gases of CF$_4$ and SF$_6$ with different concentrations were introduced into the plasma chamber at the ICP power of 600 watt, RF power of 200 watt, chamber pressure of 20 mTorr, and total gas flow of 45 sccm (standard cubic centimeter per minute). Figure 3.3(a) shows the silicon etch rate and normalized side etch width, $R$, which is defined as $R=(\text{side etch width/etch depth})$. The $R$ value is an indication of anisotropy in the etching process. The variable is gas concentration. The silicon etch rate exhibited its maximum value of 260 nm/min at CF$_4$/(CF$_4$+ SF$_6$) concentration of 0%, and normalized side etch width became a minimum at 67% CF$_4$/(CF$_4$+ SF$_6$). The result indicates that increasing the SF$_6$ concentration can etch silicon at a higher rate but it also produces a larger undercut. On the other hand, increasing CF$_4$ concentration results in the decrease in both the sidewall etching and the etch rate. These results are in good agreement with those of a previous study reported by Takahashi (2000) [23] and Ujiie et al (2000) [24]. As they mentioned, the prevention of the sidewall etching with CF$_4$ can be explained by protecting the pattern sidewall with carbon decomposed from CF$_4$. Even with the CF$_4$/(CF$_4$+ SF$_6$) concentration of 67%, vertical sidewalls could not be achieved.

The plasma-assisted etching uses a gas glow discharge to dissociate and ionize relatively stable molecules in feed gas in order to form chemically reactive and ionic species [25]. Its main chemical reactions are spontaneous etching and ion-assisted reaction. Spontaneous etching
normally takes place between long-lived radicals and surface atoms on the sidewall and the bottom, forming isotropic profiles. On the other hand, ion-assisted reaction only occurs on the bottom, enhancing reactions between surface atoms and adsorbed species. Therefore, in order to obtain highly anisotropic etching, the spontaneous reaction caused by the radicals should be dramatically suppressed by reducing the reaction probability and the incident flux of atomic radicals. There are two methods that reduce the sidewall etching. One of them is the low

![Figure 3.3: Silicon etch rate and normalized side etch width, $R$, as a function of CF$_4$/($CF_4$+SF$_6$) concentration (a) and typical SEM images after etching with 0%, 50%, and 67% CF$_4$/($CF_4$+SF$_6$) concentration.](image-url)
temperature or cryogenic assisted etching method. The reaction rate increases with the substrate temperature so that lower temperatures yield lower side etch rates. Since the ion-assisted reaction dominates at the bottom surface, bottom etch rate is rarely affected by this method. The low temperature or cryogenic plasma-assisted etching should have extremely small side etching without reducing high etch rates and high selectivity. This process, however, requires a cryogenic cooling system in order to decrease the substrate temperatures below 0 °C. The other is sequentially alternating passivation assisted etching method.

In an effort to achieve near vertical sidewalls in the plasma etching process, sequentially alternating etching with the CF$_4$ and SF$_6$ gases, which are used as the passivation and etching gases, respectively, was tested. When a gas (CF$_4$ or SF$_6$) flow rate changes, the other is fixed at 45 sccm. One cycle consists of passivation for 150 s and etching for 30 s. Four cycles were totally carried out at an ICP power of 600 watts, RF power of 200 watts, and chamber pressure of 20 mTorr. Figure 3.4(a) shows the silicon etch rate as a function of gas flow rate. Compared to the use of pre-mixed gases as shown in Figure 3.3(a), both silicon etch rate and normalized side etch width $R$ decreased. The silicon etch rate exhibited its maximum value of 61 nm/min at SF$_6$ flow rate of 45 sccm. This result indicates that silicon etch rate increases with increasing SF$_6$ flow rate. On the other hand, silicon etch rate was expected to become a minimum value at CF$_4$ flow rate of 45 sccm but it became a minimum value of 52 nm/min at CF$_4$ flow rate of 30 sccm. Increasing the CF$_4$ concentration does not always increase the surface passivation. It seems that a high CF$_4$ flow rate increases the silicon etch rate. Occasionally, CF$_4$ has been used to etch silicon after mixing with O$_2$ [23]. Also, CF$_4$ is not perfect for the passivation gas because it forms F and CF$_3$ radicals by ionization and dissociation. Commonly, deposition of a fluorocarbon polymer (nCF$_2$) is known to be more effective as the passivation layer [26].
Figure 3.4  Silicon etch rate as a function of gas flow rate (a) and typical SEM images (b-g). An alphabet in the graph is identical to each SEM image.
3.3.2 High aspect ratio silicon stamp fabrication

Imprint stamps with microposts of various diameters down to 1.5 µm were fabricated in silicon using photolithography and DRIE. The DRIE process was performed using an inductively coupled plasma machine (Surface Technology Systems) at the Micro Electronics Research Center, Georgia Institute of Technology. The gas chemistry and the power used were SF$_6$:O$_2$:C$_4$F$_8$=130:13:100 sccm and 600 W, respectively. The stamp structures were 3-12 µm deep. Examples of the stamp structures are shown in Figure 3.5. The SEM images show that the stamp structures have almost vertical and scallop-like sidewall profiles because the DRIE process involved alternating etching and sidewall passivation cycles. It was called the Bosch process and can maintain a high degree of anisotropy etching. Also, the depression or crater formation on the posts was observed in Figure 3.5(d). During photolithography, as the pattern size is close to the practical resolution limit, the light diffraction becomes significant. This causes an extra dose of UV light in the central area of the pillar structure, resulting in such cavity structures upon DRIE [27]. The fill factors (defined as the ratio between the volume to be filled by the polymer and the total volume in a local point on the stamp surface) for the stamp pillar structures shown in Figure 3.5(a), (c), and (e) are 0.93, 0.99, and 0.96, respectively. For the stamp structure with a higher fill factor, the polymer filling is faster, but stamp bending also increases during the molding process [28].

The prepared silicon stamps by DRIE were treated with a fluorinated silane prior to using them as mold in NIL. It is necessary to reduce the surface adhesion between the stamp and polymer resist during demolding. Generally, when the pattern size in a stamp decreases, the adhesion increases. When the size of a stamp becomes larger, it also increases because of the increased surface-to-volume ratio. In this experiment, C$_{10}$H$_4$Cl$_3$F$_{17}$Si ((heptadecafluoro-1,1,2,2,-
tetrahydrodecyl)-trichlorosilane) was used as a silane molecule. It consists of a head group with three chlorosilanes and a long fluorinated carbon chain. Upon deposition, the chlorosilane group in the molecule bonds with hydroxylated silicon surface, leaving volatile HCl gas. This results in the fluorinated carbon tail group facing at the surface side [27]. The fluorinated tail group

Figure 3.5 SEM images of silicon stamps with posts of 9 µm (a), 1.5 µm (c), and 3 µm (e) diameter with the fill factors of respectively 0.93, 0.99, and 0.96 produced by photolithography and DRIE.
renders the surface hydrophobic, so that the silicon surface has low surface energy. For hydroxylated silicon surface, the silicon stamp was treated with oxygen plasma for about 10 s after cleaning with acetone, isopropanol, and deionized water. The result of the silane coating was determined by water contact angle measurements. The water contact angle was typically increased from 40° to more than 110°.

### 3.3.3 Freestanding SU-8 membrane fabrication in a small area

Figure 3.2(b) shows the process scheme for producing freestanding membranes with perforated micro- and nanopores in SU-8. The key feature of this process is to use a double resist layer for NIL, which was spin-coated sequentially: first using the lift-off resist (LOR) as a sacrificial layer and then a negative photoresist SU-8 as the active membrane layer. A key challenge for the membrane fabrication was to select appropriate polymer materials that allow imprinting of the micropore structure with good replication fidelity. At the same time the material chosen should have enough mechanical stability to be freestanding and sustainable in microfluidic environments. SU-8 was chosen as the membrane layer because it is the resist most widely used for high aspect ratio microstructuring in the LIGA process (Lithographie (Lithography), Galvanoformung (Electroplating), Abformung (Molding)). After cross-linking by UV curing, high mechanical stability against an external stress such as pressure-driven flow in microfluidic applications is expected.

In order to define micropore structures in the SU-8 layer, a modified NIL process that combines thermal imprinting with a subsequent UV-curing of the molded SU-8 resist was employed. In the determination of imprinting parameters for SU-8, consideration should include the hard-baking temperature in addition to the glass transition temperature ($T_g$) for the uncured
SU-8 (~60 °C) [29]. Since the hard-baking temperature of SU-8 is ~150 °C, the resist flow at this temperature will be impeded by partially cured SU-8. On the other hand, imprinting at a lower temperature also hinders the motion of resist due to an increased viscosity. Initially, a thermal imprint process at an imprint temperature of 135 °C was used, which is just below the hard-baking temperature of SU-8, at 4 MPa for 20 min in order to maximize the resist flow. UV curing of SU-8 was performed for 10 s after the stamp was removed from the imprinted substrate at 60 °C [27]. Uniform and ordered pore patterns are seen under these imprint conditions but sagging around pores was observed, as shown in Figure 3.6. It is considered that the replication fidelity was not as good as that for thermoplastic polymers such as PMMA and PC due to an increased mechanical strength of the resist. Also, some SU-8 resist was transferred to the silicon stamp surface after demolding because of an increased adhesion attributed to the highly negative charge of SU-8 and its partial curing.

Nonetheless, these parameters can be used for small area patterning and it was used for imprinting of SU-8 with a sacrificial layer. For this experiment, the height of the pillars in the stamp was chosen to be slightly larger than the thickness of the SU-8 layer to avoid residual SU-
8 at the bottom of the imprinted holes. The residual layer thickness after NIL was low in the range of 0-200 nm depending on the ratio of stamp height to initial SU-8 layer thickness used. Even though we sometimes achieved perforation of micropores in the membrane without window opening process, the subsequent O₂ RIE process for 1 min ensures the perforation of micro- and sub-micrometer pores to the sacrificial layer. Figure 3.7 show the plot of an etched thickness of SU-8 as a function of RIE etching time. The residual layer can be removed by RIE
process with an etching rate of \( \sim 250 \text{ nm/min} \) etching rate but the roughness of membrane surface was increased (see Figure 3.7(c) and (d)). However, the roughness might be reduced by using mixed O\(_2\) with CF\(_4\) gas. According to Hu et al, the introduction of F in CF\(_4\) significantly reduces the roughness of the etched surface and increased the etch rate [29].

![Figure 3.7](image)

Figure 3.8 SEM images of 1.5 \( \mu \text{m} \) diameter pores imprinted on SU-8 using PMMA as sacrificial layer (a) and freestanding SU-8 membrane after releasing from silicon substrate (using LOR as sacrificial layer) (c). (b) and (d) are close up view of (a) and (c), respectively.

Initially, PMMA with a low molecular weight was used as the sacrificial layer because PMMA can be easily dissolved in acetone. Figure 3.8(a) and (b) shows SEM images of cured SU-8 membrane with 1.5 \( \mu \text{m} \) diameter pores after attempting to dissolve PMMA sacrificial layer with an acetone for 3 hours. Even after 1 day, the SU-8 membrane layer was not fully released.
The reason seems to be insufficient solubility of PMMA to acetone and insufficient transport of acetone through the micropores [27]. However, when LOR was used as the sacrificial layer, SU-8 membranes was completely released from the silicon substrate. A fully released SU-8 membrane with perforated micropores was obtained by dissolving the sacrificial layer with a MF319 solution. It took ~2 hours to complete the lift-off process with 1 µm thick LOR layer. Examples of the released SU-8 membrane are shown in Figure 3.8(c) and (d). The membrane was completely perforated, having 9 µm diameter pores which were almost identical to the size of silicon stamp pillars. However, sometimes an unexpected pore around defined pores was observed. It was made because SU-8 resist was not completely filled during imprinting.

![SEM images of 1.5 µm (a) and 500 nm (c) diameter pores imprinted on SU-8. (b) and (d) are close up view of (a) and (c), respectively.](image-url)

Figure 3.9 SEM images of 1.5 µm (a) and 500 nm (c) diameter pores imprinted on SU-8. (b) and (d) are close up view of (a) and (c), respectively.
With the process developed, the smallest pore structure with 1.5 µm diameter was repeatably achieved when the stamp having pillars with 2 µm diameter was used, as shown in Figure 3.9(a) and (b). Overall, the resulting pore size was slightly smaller than the diameter of pillars in the stamp. However, sometimes the pore diameter was significantly reduced to 500 nm and sagging around the pores occurred, as shown in Figure 3.9(c) and (d). This can be explained by the relaxation (or reflow) of the uncured SU-8 just after demolding. The demolding was carried out at 60 ± 5 °C [27] which was close to T_g of SU-8. Therefore, SU-8 was still viscous state and it makes the reflow of uncured SU-8. In Chapter 7, the reflowing will be explained in detail. Also, the small pore had less sag than the larger pores shown in Figure 3.6. This can be attributed to different filling behavior for structures with different diameter/period ratios [27].

3.3.4 Freestanding SU-8 membrane fabrication in a large area

When a 10.16 cm stamp fully covered with micropillars was used, clean demolding could not be obtained with the imprint conditions mentioned above. It was observed that the adhesion of the imprinted SU-8 layer to the silicon stamp surface was significantly higher than that of PMMA or PS, which can be attributed to the highly negative charge of SU-8. Thermal stress generated during cooling also contributed to the increase in the stress during demolding. In order to overcome this problem, we changed the imprint process in two ways: (1) reduced the imprint temperature while increasing the imprinting pressure and (2) UV curing was performed before demolding. The former significantly lowers the thermal stress generated during cooling. The latter both decreases the adhesion between the stamp and the imprinted SU-8 substrate and prevents dimensional changes of the SU-8 patterns by relaxation after demolding. After imprinting at 55-85 °C and 5 MPa for 2 min, the temperature was reduced to 50 °C before the
sample was exposed to UV light for 10 s through the quartz substrate to cross-link the imprinted SU-8 layer. Demolding was then performed at 40 °C and then the substrate was subsequently baked at 95 °C for 5 min to complete the cross-linking of the molded SU-8 layer.

Figure 3.10 shows optical micrographs of the imprinted SU-8 patterns for different temperatures, while the pressure and imprint time were kept constant (5 MPa and 2 min). At 55 °C the edge of pores was not sharp due to insufficient resist flow at this temperature, as shown in Figure 3.10(a). Also, some parts were detached during demolding. At 65-85 °C complete filling was observed, as shown in Figure 3.10(b)-(d). However, some parts of the resist were detached during demolding and this problem becomes significant as the demolding temperature increased. The optimal temperature was to be 65 °C in terms of sufficient resist

![Image](image_url)

**Figure 3.10** Optical micrographs for temperature varying study of SU-8 imprinted at 5 MPa for 2 min. (a) 55 °C (b) 65 °C (c) 75°C, and (d) 85°C
filling with the least demolding problems. These conditions are comparable to those used in a reversal imprint process (40-85 °C and 1-5 MPa) reported by Hu et al (2006) [29]. With these process parameters, we could achieve large area, released SU-8 membranes up to 10.16 cm diameter, as shown in Figure 3.11(a). The membrane with 3.6 µm thickness was fully covered with perforated micropores with 3 µm diameter. Figure 3.11(c)-(d) show SEM images for top and bottom surfaces of the released membrane, respectively, taken after placing on a dummy wafer. The images confirm the perforation of the micropores through the membrane.

Figure 3.11 (a) Photograph of a large area, freestanding SU-8 membrane with a 10.16 cm diameter and fully covered with perforated 3 µm pores and 3.6 µm thickness; (b) its SEM image after attaching the membrane on a dummy Si wafer; SEM images of (c) top (contact with the mold) and (d) bottom (contact with the substrate) view of the SU-8 membrane with 3 µm pores.
3.4 Conclusions

A low cost and flexible method using all parallel processes to produce large area, fully released polymer membranes containing perforated micropores in SU-8 was developed. For the fabrication of the membrane, a combination of NIL with a sacrificial layer technique was employed.

The fabrication of the SU-8 membrane with pore sizes down to sub-micrometer was only demonstrated due to the limitation of our process to produce nanoscale stamps. However, considering sub-10 nm resolution of NIL, the developed process can also be used to produce membranes with nanoscale perforated pores. The key challenges include fabrication of nanoimprint lithography stamps with high aspect ratio nanopillars and high aspect ratio imprinting, which are required to achieve enough mechanical strength to be freestanding after complete release of the membrane. With the practical limitation of high aspect ratio imprinting considered, such membrane structures with perforated pores of ~100 nm diameter will be achievable using this process. Ultimately, sub-100 nm pores may be achievable by employing a subsequent process to further reduce the pore size such as polymer grafting.

3.5 References


Chapter 4. A Microfluidic Platform with Micropore Membranes for In-Situ Formation of Lipid Bilayers

4.1 Introduction

In biology, numerous studies on electrophysiological properties for ion channel proteins of cells have been performed using black lipid membranes (BLMs). BLMs are artificial lipid bilayers formed in a perforated aperture and mostly formed in an aperture with relatively large diameter in the range of 50-200 μm [1-6]. However, BLMs formed in large apertures are mechanically fragile and easily broken by small mechanical and electrical disturbances [7]. The stability of BLMs and the reproducibility of electrical measurements through BLMs have been shown to improve by reducing the aperture size [8-10]. Mayer et al. demonstrated that the noise for recordings of ion fluxes through ion channels in BLMs formed in apertures smaller than 40 μm was reduced to 0.4 pA rms at 4.3 kHz band width and that BLMs were stable at voltages up to 460 mV [8].

In addition to the use of small apertures, integration of BLMs formed in microapertures into a microfluidic system offers further improvement [10-13]. Mach et al. used a microfluidic device allowing the formation of a planar lipid bilayer across a micron-sized aperture in a glass slide sandwiched between two polydimethylsiloxane (PDMS) channels [10]. They demonstrated that the miniaturized system allows low noise recordings comparable to open head-stage noise, fast precision perfusion on each side of the membrane, and the use of nanoliter analyte volumes. In addition, the use of a microfluidic system has the advantages of preventing solvent evaporation and contamination during the analysis, which could lead to erroneous measurement results. Therefore, the ability for high-throughput fabrication of a microfluidic system integrated with lipid bilayers formed in micro- and nanoscale pores is critical for improved
electrophysiological measurements of biosystems.

In this chapter, a simple process to integrate a freestanding SU-8 membrane into a microfluidic system is introduced, indicating the potential of the membrane to be used as a modular component in lab-on-a-chip devices. A flexible method reported in the previous chapter was used to produce the membranes with perforated pores down to sub-micrometer diameter by a combination of nanoimprint lithography and a sacrificial layer technique. The feasibility of using the membrane as a bio-platform was examined by reconstituting lipid bilayers at the micropores of the membrane surface within the microfluidic system.

4.2 Experiments

4.2.1. Photomask Design

A photomask was used for patterning negative photoresist coated on silicon wafer in order to make microfluidic molds. Two different microchannel structures were designed in nine separated areas (2 cm × 2cm) on a photomask. The first structure (having one inlet) occupies six areas and the other one (having two inlets) occupies the rest of the mask. In both cases the width

Figure 4.1 AutoCAD design for the photomasks in order to fabricate microfluidic channels.
and length of the main channel are 150 μm and 6 mm, respectively. Figure 4.1 shows AutoCAD pattern for the photomask.

The pattern AutoCAD file was converted to a standard format compatible with an optical pattern generator (Mann 3600 photo repeater, GCA Co.) in CAMD. A soda-lime photomask blank (Telic Co.), which was coated with AZ1500 resist on Cr layer was irradiated with UV light according to the designed pattern. The exposed resist was removed by developing for 90 s in 351 developer (Shipley Co.) mixed with deionized water (3:1 volume ratio of developer to deionized water). The mask was subsequently kept in a deionized water bath for 5 min and then immersed in a Cr etch bath for 60 s. Etching of the Cr layer resulted in opening the window in the glass substrate exposing the glass beneath. A subsequent deionized water immersion was performed for 5 min followed by an acetone bath treatment for 5 min to remove the resist layer. The mask was treated briefly to O₂ plasma for about 10 s to ensure complete removal of any resist residue. The photomask was washed in flowing deionized water to remove acetone and blow dried with N₂.

4.2.2. Assembly of the SU-8 membrane into a microfluidic system

In order to fabricate an integrated microfluidic platform, an SU-8 membrane with 3 μm diameter pores (see Figure 3.11) was sandwiched between two crossed PDMS microchannels. In order to fabricate simple microfluidic channels, SU-8 negative photoresist was patterned on silicon via photolithography. A layer of SU-8 (SU-8.100, MicroChem Co.) resist was spin-coated at 1500 rpm for 60 s, followed by a two-step baking process at 65 °C for 10 min and 95 °C for 10 min, respectively. A thickness of pre-exposed SU-8 was controlled to be 150 μm with a flycutter (Optimum 130, Precitech Inc.), followed by post fly-cutting baking at 65 °C for 10 min.
Photolithography was done with the designed photomask in a UV exposure station (UL7000-OBS Aligner and DUV exposure, Quintel Co.) at a class 100 cleanroom. The exposed wafer was then developed with SU-8 developer (MicroChem Co.), followed by washing with isopropanol and deionized water.

The SU-8 pattern on the silicon wafer (see Figure 4.2) was replicated by casting PDMS (10:1 mass ratio of silicone elastomer to curing agent). After curing overnight at room temperature, the PDMS replica was peeled off from the master. One of the PDMS replicas having a microchannel was gently stamped onto a substrate spin-coated with the PDMS curing agent, which is used as an adhesive material between the two PDMS replicas [14]. With the other PDMS replica, fluidic inlets and outlets were formed using a hole-puncher. The two PDMS replicas were then bonded at 70 °C for 1 hour in a vacuum oven after placing the membrane between them. Finally, PEEK tubes (0.178 mm inner diameter, 0.793 mm outer diameter, Upchurch Scientific Inc.) were connected to the punched holes without using any glue.

Figure 4.2 A photograph of SU-8 microfluidic patterns on silicon wafer. It was replicated by casting PDMS.
4.2.3. Lipid adsorption on SU-8 membrane

Before exposing the SU-8 membrane with a lipid solution, some of the membrane surfaces was treated with poly(L-lysine)-grafted-polyethylene glycol (PLL-g-PEG) which is known to significantly reduce non-specific adsorption of proteins at solid surfaces. We first synthesized PLL-g-PEG following the synthetic path described in [15]. Prior to the PLL-g-PEG treatment, the SU-8 membrane surface was activated by oxygen plasma for 10 s. A 2 µL PLL-g-PEG solution was then applied on the patterned area for some of the membranes. The treated membrane was subsequently placed in a covered glass Petri dish. A cotton ball, wet with deionized water, was kept alongside the membrane in the covered Petri dish in order to prevent the PLL-g-PEG solution from drying out. After 10 min, the PLL-g-PEG treated membrane sample was subsequently washed in a 4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic (HEPES, Sigma Aldrich) buffer (pH = 7.4) and dried in a clean environment. The contact angles of SU-8 surface before and after oxygen plasma, and after the PLL-g-PEG treatment were 66 ± 1.4°, 39 ± 1.7°, and 28 ± 0.4°, respectively (see Figure 4.3).

Figure 4.3 Water contact angle of SU-8 surface (a) before and (b) after oxygen plasma, and (c) after the PLL-g-PEG treatment. It were 66 ± 1.4°, 39 ± 1.7°, and 28 ± 0.4°, respectively.
For lipid adsorption experiments, 20 mg of phosphatidylcholine dissolved in chloroform (1,2-Dipalmitoyl-sn-Glycero-3-Phosphocholine (DPPC), Avanti Polar Lipids) was mixed with chloroform (Purity=99.9%, Fisher Scientific) as a volume ratio of 1:100. 0.02 mg of Rhodamine B-labeled phosphatidylethanolamine dissolved in chloroform (1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine-N-(lissamine rhodamine B sulfonyl)) (Avanti Polar Lipids) is then added to 1 mL of lipid solution for fluorescent observation. After dispensing 2.5 µL of the stained solution, excessive lipid vesicles on the membrane were washed off with deionized water for 2 min and the membrane was inspected under a fluorescence microscope (DM2500, Leica).

4.2.4. Lipid bilayer formation within microfluidic system

Lipid bilayers were formed at micropore sites of the SU-8 membrane within the assembled microfluidic platform following the sequence used by Suzuki et al [16]. Basically, this method utilizes the fact that a lipid bilayer is spontaneously formed when a thin lipid solution is confined between two aqueous buffers due to the electrostatic interaction between the lipids and the buffers. In order to realize the buffer/lipid solution/buffer configuration, firstly, 0.1 M potassium chloride (KCl) buffer obtained by dilution of 1 M KCl solution (BioUltra, Sigma-Aldrich) is filled into the bottom channel at a flow rate of 2 µL/min. The lipid solution is then introduced to the upper channel and squeezed out with air at a flow rate of ~0.1 µL/min. For the lipid solution, 20 mg of phosphatidylcholine dissolved in chloroform (1,2-Dipalmitoyl-sn-Glycero-3-Phosphocholine (DPPC), Avanti Polar Lipids) is added to 1 mL of n-decane (99%, Alfa Aesar). Then, 0.02 mg of Rhodamine B-labeled phosphatidylethanolamine dissolved in chloroform (1,2-Dipalmitoyl-sn-Glycero-3-Phosphoethanolamine-N-(Lissamine Rhodamine B Sulfonyl), Avanti Polar Lipids) is added to 1 mL of lipid solution for fluorescent observation.
Finally, another buffer solution was gradually introduced into the upper channel at a flow rate of \(~0.1\ \mu\text{L/min}\) to complete the formation of lipid bilayers. For fluorescent observation, an inverted optical stage (IX-81, Olympus) with a green excitation filter (U-MWG2, Olympus) was used.

4.3 Results and discussion

4.3.1. Integration of the SU-8 membranes into a microfluidic system

Figure 4.4 (a) shows a schematic image of how to integrate the released SU-8 membrane into a modular microfluidic device. Since the membrane is mechanically stable enough to be freestanding without crumbling, it can be used as a modular component to build a fluidic system. With this idea, the SU-8 membrane with perforated micropores was sandwiched between two PDMS microfluidic chips and the entire device was bonded by using the PDMS curing agent as glue and subsequent heating. The microchannels in the two PDMS chips were aligned to be perpendicular to each other. One advantage of such a crossed orientation is that only micropores in the membrane which are located within the overlapped area between the two microchannels will be actively involved in the transportation of substances through the pores. Therefore, the

Figure 4.4  (a) a schematic image for assembly of the freestanding SU-8 membrane with perforated pores into a microfluidic system (b) a photograph of a microfluidic system in which a freestanding SU-8 membrane was sandwiched between two PDMS chips with microchannels.
number of active pores can be controlled simply by using microchannels of different widths. This will also alleviate the requirement of high accuracy alignment in assembling the microfluidic system. The fabricated PDMS microfluidic device integrated with about 3.6 µm thickness of SU-8 membrane (see Figure 3.11) is shown in Figure 4.4(b). This process is similar to those reported previously where a thin polycarbonate track-etched membrane has been integrated between two PDMS layers with embedded microchannels [17, 18]. Here, we used microchannels of 150 µm width. The membrane was successfully integrated with the microchannels without sagging and folding.

Using the integrated microfluidic system, a leak test was performed with both liquid and gas. Air and deionized water were simultaneously introduced into the upper and bottom channels, respectively (vertical and horizontal channels in the figure). When the outlet of bottom channel was blocked, deionized water first filled the bottom channel and then flowed into the upper channel through the micropores. Since the outlet of upper channel was open, deionized water can easily flow within upper channel and flowed out to the outlet of upper channel. Air fragment was observed on this account, as shown in Figure 4.5(b). Meanwhile, when the both flow was stopped, the inlet of bottom channel was open, and the outlet of upper channel was closed, air in the upper channel with residual pressure flowed into the lower channel through the micropores in the SU-8 membrane, as shown in Figure 4.5(d). For flow of both air and deionized water through the micropores, the membrane was intact, which corroborates that the SU-8 membrane represents a stable modular fluidic interface for microfluidic systems over the fragile Si₃N₄ or SiO₂ membranes (e.g. usually, the micro- and nanopore membranes fabricated by micro- and nanofabrication techniques have been formed mostly in a thin Si₃N₄ or SiO₂ layer at hundreds of nanometers in thickness). Even though cured SU-8 is often considered as brittle, the SU-8
membrane allows for larger strain (or more flexible) than Si₃N₄ or SiO₂ membranes because of the availability of thick membranes in the micrometer range via spin-coating.

Figure 4.5 A schematic and micrograph of a leakage test with deionized water and air with the microfluidic system in Figure 4.4(b): (a) deionized water flowed into the upper channel through the micropores from the bottom channel (the upper and bottom channels are vertical and horizontal channels in the figure, respectively), (c) air in the upper channel with residual pressure flowed into the lower channel through the micropores.
4.3.2. Lipid adsorption on the SU-8 membranes

The feasibility of using the SU-8 membrane with perforated pores for a platform for BLMs was examined by studying the adsorption behavior of lipids to the membrane surface because precise location of lipids at the pore sites is the first step for the formation of lipid bilayers. For the experiment, a membrane with 3 µm pore diameter was used. Prior to the lipid adsorption, some of the SU-8 membrane was treated with a PLL-g-PEG solution, which has been known to prevent non-specific adsorption of many biopolymers at biofunctional surfaces [19, 20]. Figure 4.6 shows fluorescence micrographs after exposure of the differently treated SU-8 membranes to a lipid solution. For the SU-8 membrane with well-defined micropores (see Figure 4.6(a) and (b)), lipid vesicles preferentially adsorb at the pore sites in the membrane. This may

![Fluorescence micrographs after exposure of the SU-8 membrane with 3 µm pores to a lipid solution. (a), (b) show vesicles preferentially adsorbed at well-defined micropores, while (c), (d) display defected micropores.](image)

Figure 4.6  Fluorescence micrographs after exposure of the SU-8 membrane with 3 µm pores to a lipid solution with ((b), (d)) or without ((a), (c)) the PLL-g-PEG treatment with well-defined micropores (a), (b) and defected micropores (c), (d).
be accounted for as the low entropy state at the pore sites. When the membrane surface was treated with PLL-g-PEG prior to the lipid adsorption, the selective adsorption at the pore sites was still observed but the fluorescence signal becomes significantly weaker, indicating that the PLL-g-PEG treatment reduces the number of lipid vesicles adsorbed at the pores. The adsorption behavior is dramatically changed when unintended surface roughness exists in the SU-8 membrane surface due to failed imprints (see Figure 4.6(c) and (d)). A strong fluorescence signal emanates from the defect sites, which is attributed to non-specific adsorption of lipid vesicles at defect sites. Even though details on the relationship between instability of the lipid layer and surface defects could not be extracted, this result implies that achieving good imprinted patterns is a prerequisite for using the polymer membrane as a component in microfluidic platforms for the electrophysiological study of membrane proteins of cell membranes.

Normally, surface properties such as chemistry and hydrophobicity play important roles in the adsorption of biomolecules. The selection of proper materials and surface treatments are critical for many bio-applications [21, 22]. In the current experiments, it found that the SU-8 surface without imprint defects does not allow for adsorption of lipid vesicles irrespective of the PLL-g-PEG treatment, which is an important surface property to achieve selective lipid adsorption at the pore sites. The SU-8 can potentially be used as substrate material to fabricate mechanically stable bio-platforms with BLMs for biophysical studies.

4.3.3. Lipid bilayer formation on SU-8 membrane within a microfluidic system

After the confirmation of selective adsorption of lipids at the pore sites in the SU-8 membrane, the formation of lipid bilayers within a microfluidic system were examined, which is important for developing an automated multichannel system for the study of membrane proteins
In order to produce a configuration where a thin lipid solution is confined between aqueous solutions, known to spontaneously form a lipid bilayer, the first step involves filling the bottom channel with KCl solution. During this process, some air bubbles can be observed. Lipid solution was then filled into the upper channel and squeezed out with air. KCl solution was finally filled into the upper channel to form a lipid bilayer.

Figure 4.7  A schematic of lipid bilayer formation within microfluidic system and their micrograph images: (a) KCl solution was filled into the bottom channel. During this process, some air bubbles can be observed (shown as figure 4.7(b)), (c) lipid solution was filled into the upper channel and squeezed out with air, and (e) KCl solution was filled into the upper channel in order to form lipid bilayer.
microfluidic channel with buffer solution (see Figure 4.7(a)). At this stage, the buffer remained at the front of the micropores and did not flow out into the upper channel due to surface tension. Then, the lipid solution was introduced to the upper channel and then squeezed out by flowing air (see Figure 4.7(c)). At this moment the PDMS became swollen due to chloroform absorption but it was slowly released during the squeezing step with air and the final step. On the other hand, no significant swelling or dissolution of the integrated SU-8 membrane by introducing chloroform into the microchannels. Fluorescence signal emanates from both the micropores and non-structured surface. Only the lipids at the micropores located at the crossed area between the two microchannels are interfaced with the first buffer solution. The corresponding fluorescence micrograph is shown in Figure 4.8(a). Finally, the buffer solution was filled into the upper channel (see Figure 4.7(e)). This step removes excessive and non-specifically adsorbed lipids existing in the upper channel and, more importantly, allows for the buffer/lipid solution/buffer configuration at the micropores located in the crossed area by two microchannels to form lipid bilayers. In spite of an overall decrease in the fluorescence intensity, weak fluorescence signals emanate from the micropore sites at the crossed area indicating the formation of lipid bilayers (see Figure 4.8(b)). Even though the lifetime of the lipid bilayer in the membrane pores were not been measured, the stability of the lipid bilayer may be influenced by pore size and geometries (vertical or tapered sidewall), and defects in the membrane, which needs to be further exploited before applying the lipid bilayer for biophysical studies of biosystems.

An electrical measurement between the upper and lower microchannels in order to independently confirm formation of a lipid bilayer was performed. However, the results indicate direct conduction between two electrodes, as opposed to the existence of a capacitor which is the case when the lipid bilayer seals the two microchannels completely. This has been attributed to
the imperfection of the process, leaving some pores not perfectly sealed by the lipid bilayers. Even a single pore that is not perfectly sealed will be used as a path for electrical conduction. Indeed, missing fluorescence signal at some micropores indicated by red triangles in Figure 4.8(b) was detected. Therefore, despite the advantage of low-cost and high throughput for the membrane fabrication, the process needs to be further improved to achieve giga ohm seal with lipid bilayers for all the active micropores in order to be able to implement the microfluidic system with embedded BLMs to biophysical studies of membrane proteins. One approach for this improvement may be the use of a single micropore at the active membrane area instead an array of micropores as used in this study.

4.4 Conclusions

An elegant method using the porous SU-8 membrane as a well-defined fluidic interface in a modular manner for microfluidic systems has been shown, suggesting that such membrane architecture can potentially be used as a component for modular lab-on-a-chip devices. By
developing a route for selective adsorption of lipid vesicles at the pore sites in the SU-8 membrane and within a microfluidic system, the feasibility of using the membrane for a platform for BLMs has been demonstrated.

4.5 References


Chapter 5. Low Cost Fabrication of Conical-Shaped Nanopores in Freestanding Polymer Membranes

5.1 Introduction

Although nanoimprint lithography (NIL) can successfully fabricate structures down to 10 nm [1], it is difficult to produce perforated pores with diameters less than 100 nm in freestanding polymer membranes. Since it requires molding using a stamp with extremely high aspect ratio to achieve high mechanical stability for the membrane fully released from a substrate. In practice, there are several issues that should be addressed to successfully use nanoimprint lithography to create a freestanding polymer membrane: (1) choosing optimal materials, (2) fabricating reliable stamps with high aspect ratio structures having sub-100 nm features, and (3) improving anti-stick coatings [2]. In this chapter, we describe utilizing silicon microneedle structures as an imprinting mold that overcomes these issues, leading to fabrication of conical-shaped nanopores in a freestanding polymer membrane. Conical-shaped means that the pore on one side (base pore) is wider than the pore on other side (tip pore). The advantage of conical-shaped pores for sensing purposes is that the zone for sensing analyte species appears in and near the tip pore where the major voltage drop occurs [3, 4].

5.2 Experiments

5.2.1 Photomask Design

A photomask was used for patterning negative photoresist coated on a silicon wafer. The structures on the photomask are comprised of circles, squares, and rectangles of varying diameters, widths and periodicities. In order to fabricate single or multiple microneedles, the square (25 µm × 25 µm) structures were used as an etching mask. For a single microneedle, the
pattern was surrounded with four alignment structures (250 µm × 250 µm). For multiple microneedles, each pattern was positioned at intervals of 50 or 150 µm. It is shown in Figure 5.1. This photomask with a Cr layer as a clear field pattern was custom ordered from Advanced Reproductions (North Andover, MA).

5.2.2 Fabrication of silicon stamp with microneedle patterns

P-type silicon (100) wafers with resistivity of 1-10 Ωcm and covered with a 100 nm thick layer of silicon nitride deposited using LPCVD (Low-Pressure Chemical Vapor Deposition) were used in all experiments. Si stamps with microneedle patterns were fabricated using a combination of photolithography and wet etching techniques. First, a ~0.75 µm thick layer of negative photoresist (SU-8 2000.5, MicroChem Co.) was spin-coated at 1000 rpm for 45 s on the substrate which was treated with oxygen plasma in order to modify its surface and dehydrated at 110 °C. Subsequently, a pre-exposure bake was performed at 95 °C for 60 s. Photolithography was done with a custom designed photomask (Advance Reproductions Co.) in a UV exposure
station (UL7000-OBS Aligner and DUV exposure, Quintel Co.) at a class 100 cleanroom. UV exposure was carried out at 80 mJ/cm$^2$ and post exposure baking was followed at 95 °C for 60 s. The wafer was then developed in SU-8 developer solution (MicroChem Co.) for 60 s, followed by washing with isopropyl alcohol (IPA) for several minutes and drying with N$_2$ gas. Any residual layer in the recessed area of the resist patterns was removed by oxygen plasma for about 10 s. Then, the nitride layer on the substrate was patterned with square etch masks using a plasma etch process at an RF power and chamber pressure of 150 watt, 20 mTorr, respectively. The flow rate of the SF$_6$ gas was 45 sccm (standard cubic centimeter per minute).

The wet etching was performed in a glass beaker filled with 35 wt% KOH solution at 80 °C without isopropyl alcohol (IPA). The etching solutions were prepared by dissolving KOH pellets (85%, Fisher Scientific Inc.) in deionized water. Subsequently, the barrier nitride layer on the silicon surface was removed by hydrofluoric acid (48%, EMD Chemicals Inc.) and was cleaned in acetone, isopropyl alcohol and deionized water for 5 min each. Prior to imprinting, the silicon surfaces were treated with a fluorinated silane in the vapor phase to reduce adhesion to the resin.

**5.2.3 Fabrication of SU-8 membrane having conically shaped nanopore**

To fabricate the SU-8 membranes, NIL was combined with a sacrificial layer technique. A silicon substrate was spin-coated with a double resist layer (see Figure 3.2(b)). The various thickness of LOR layer (PMGI SFG 2S, LOR 3B and LOR 7B, MicroChem Co.) was first spin-coated at different spin rates for 60 s as a sacrificial layer, then baked at 150 °C for 5 min. On the LOR layer, an about 5-6 µm thick SU-8 (SU-8.5, MicroChem Co.) layer was spin-coated at 1500 rpm for 60 s, followed by a two-step baking process at 65 °C for 1 min then at 95 °C for 1 min.
NIL was then performed in the resist layer with a commercial nanoimprinter (Eitre®, Obducat). Finally, the membrane with perforated conical-shaped nanopores was released by dissolving the sacrificial LOR layer with a MF319 solution.

### 5.2.4 Current-voltage measurement

The microfluidic system integrated with the SU-8 membrane (described in Chapter 4) used as a platform to study transport of ions through the nanopores. For this experiment, the microfluidic system was filled with 0.1 M concentrations of KCl solution (1 M KCl, Fluka) buffered with 10 mM Tris (Tris(hydroxymethyl)aminomethane) and 1 mM EDTA (Ethylenediaminetetraacetic acid) (100× Tris-EDTA buffer solution, Fluka) to a pH of 8. It was then connected to an Axopatch 200B (Molecular Devices Corp.) patch-clamp amplifier. Current-Voltage (I-V) curves associated with ion transport through the nanopore were recorded in voltage-clamp mode using a low-pass Bessel filter with 2 kHz bandwidth. The signal was digitized with a Digidata 1322A analog-to-digital converter (Molecular Devices Corp.) at a sampling frequency of 10 kHz, and viewed with the Clampex 9.2 software (Molecular Devices Corp.). 2 min ion current measurements were recorded at constant voltages sweeping over a potential range ±100 mV with 10 mV increments. I-V curves were recorded in triplicated scanning from -100 mV to +100 mV and in triplicate from +100 mV to -100 mV. Also, in order to remove small offsets between each runs, all curves were adjusted to zero current at zero volts.

Two Ag/AgCl electrodes, used to control the applied potential and measure the ionic current flowing through the nanopore during experiments, were used to minimize the effect of electrode polarization. Ag/AgCl electrodes were prepared by bleaching 1 mm diameter, 99.99% pure silver wire (Sigma-Aldrich) with 6% sodium hypochlorite (NaOCl) solution for 30 min.
Prior to bleaching, the wire was insulated by covering it with PTFE/FEP dual-shrink tube (Small Parts Inc., 0.060 inch inner diameter and 0.028 inch wall thickness), which is highly resistant to most chemicals. The working Ag/AgCl electrode was then placed at the base (the larger opening) of a conical-shaped nanopore, which was used to apply a given potential difference. The other electrode was placed at the tip (the narrow opening) of a pore, which was held at ground.

5.3 Results and Discussion

5.3.1 Fabrication of silicon stamp with microneedle shape

As mentioned in Chapter 3, the key challenges to obtain nanopores with diameter less than <100 nm in polymer membranes using the process shown in Figure 3.2(b) are the fabrication of silicon molds with high aspect ratio (>10:1) nanopillars and imprinting with the high aspect ratio molds. This is necessary because the thickness of membranes should be at least several micrometers for enough mechanical stability to be freestanding and sustainable in microfluidic situations. In order to avoid the requirement of high aspect ratio imprinting but still achieve the membrane stability, the use of silicon molds having microneedle structures with the nanoscale curvature radius for the apex is a promising alternative. Silicon microneedles have been used for biological and medical applications such as biological fluidic extraction and drug delivery. Therefore, their fabrication methods, isotropic and anisotropic dry etching and anisotropic wet etching, have been well established.

One of the methods to produce microneedles in silicon is wet chemical etching of islands with a convex-shaped mask patterns. In this process, the microneedles are developed by two planes (bottom and upper parts) according to each of the forced crystallographic zones. The lower portions of structures are limited by (221), (331), or (441) planes inclined toward the
surface (100) by 48.18°, 46.51°, and 45.87°, respectively. On the other hand, the upper portions of structures are limited by (211), (311), or (411) planes inclined toward the surface (100) at angles of 65.9°, 72.45° and 76.37°, respectively [5]. Also, the planes (221), (331) and (441) as well as (211), (311) and (411) were developed lying in directions [210], [310] and [410], respectively. Depending on etching conditions such as crystal orientation, temperature, KOH concentration and alcohol additive, however, those planes can appear or disappear (i.e. the pattern will be irregular shape) [5].

In this work, the square Si₃N₄ masks aligned along the [110] direction exhibited (311) and (100) planes of the wafer during wet etching at rates of 1.09 and 0.85 µm/min, respectively. This result agrees well with those reported by Liu et al [6] to obtain an ultra-sharp tips, having 0.78 as the experimental η (the condition for self-sharpening) value. Figure 5.2 shows optical microscope images showing the progress of the silicon etching process to form a microneedle through convex corner undercutting of the square mask. The convex corner undercutting was followed by five steps, which are dominated by the planes having highest or slowest etching rate at different cross-sectional areas [7]. The steps are (111) slope formation, (110) slope formation, (100) slope formation under the etch mask, (311) slope formation at the intersection between (100) and (111) planes, and (311) slope growth in consecutive order. Indeed, the (111) planes appeared along each side of the square masked area with resulting the mesa structure on the (100) surface. A (110) plane then appeared at the intersection between two (111) planes (see Figure 5.2 (1 min)). After 3 min, the (311) plane appeared and grew up before the needle was formed (see Figure 5.2 (9 min)). However, the (110) slope formation, (100) slope formation under the etch mask and (311) slope formation at the intersection between the (100) and (111) planes, which are mentioned in [7] were not observed because the etch rate was too fast to observe their formation.
Also, unexpected planes, which were not mentioned in [7], irregularly appeared before forming a microneedle (see Figure 5.2 (5 min)). Finally, a microneedle with a sharp tip was formed when the eight (311) planes meet on top of the frustum in a single point, thereby breaking down an inverted structure (see Figure 5.2 (after)) [8, 9].

Scanning electron microscopy (SEM) analysis of the fabricated microneedles revealed that KOH etching procedure easily yielded an array of uniform silicon microneedles having ultra-sharp apexes without using any high-end nanofabrication protocol (see Figure 5.3). A SEM
image of the tips with a 50 µm period shows that they are nearly uniform, with an average bottom diameter of 5.1 ± 0.2 µm and an average tip height of 8.3 ± 0.1 µm (see Figure 5.3(a)). SEM analysis shows that the sidewalls of the needles actually consist of two different planes; the bottom and upper planes which make an angle of 46.5° and 72.4°, respectively with (100) surface (see Figure 5.3(b)). Also, the surface intersection angle, a₁ and a₂, and the rotation of the intersection of the planes to the [100] direction of the wafer, a₃, are 126.9°, 143.1° and 18.4°, respectively (see Figure 5.3(c)). It implies that the bottom sidewalls are (33 1) planes and the
The average apex diameter is 25 ± 2 nm, demonstrating that self-sharpening has been accomplished (see Figure 5.3(d)). Moreover, its low surface roughness and conical shape make it a suitable candidate as a mold structure in terms of easy demolding. In theory, the apex diameter and its surface roughness could be reduced down to 5 nm [10] and 10 nm [11] by adjusting etching conditions, respectively.

However, the etching process is not straightforward; Inhomogeneous etching can occasionally happen. Figure 5.4 shows SEM images of the etched silicon microneedles in different locations in a 1.5 cm × 1.5 cm stamp. The microneedles at the center area were etched more than those at the edge area where the Si₃N₄ etch mask still remains on the apex of the microneedles. It can also be seen that different crystallographic planes were developed in the low portion of the microneedles. The planes shown in Figure 5.4(b) usually appear when developed in an aged KOH bath [12]. Wilke and co-worker mentioned the height of upper part decreased when an aged KOH bath was used. This means that the etch bath conditions also need to be controlled in order to obtain high index crystals (i.e. microneedle shape) by the etching. Another interesting observation is an inverted structure, as shown in Figure 5.4(b). Some inverted
structures (e.g. needle or pyramid shape) were broken slightly earlier before reaching a sharp needle tip during etching due to mechanical stress caused by water pressure.

5.3.2 Fabrication of SU-8 membrane having conically shaped nanopore

The NIL capability of a silicon needle as an imprinting mold was evaluated (see Figure 5.5). Prior to imprinting, its surface was treated with a fluorinated silane in the vapor phase to reduce adhesion to the polymer resist. NIL was performed at 65 °C, 1 MPa for 2 min using a commercial nanoimprinter which allows for both thermal and UV imprinting. A SEM image of the base pore patterns with a pitch of 50 µm shows that they are remarkably uniform, with an

![SEM images](image1.png)

(a) Shows imprinted base pore on 1 µm thick LOR. (b) Shows a magnified image of the needle array shown in (a). (c) Shows cross section view of imprinted pore on 1 µm thick LOR, and (d) Shows freestanding polymer membrane after releasing from silicon substrates.

Figure 5.5
average pore diameter of 3.6 ± 0.1 µm (see Figure 5.5(a)). SEM analysis shows that the pore has an octagon shape, similar to the circumference of silicon needles in the mold (see Figure 5.5(b)). Also, a cross-sectional SEM image displays that the pore has a cone shape with a slope angle of 72.45° and the needles seem to completely have penetrated into SU-8 layer without additional RIE process to remove residual SU8 layer (see Figure 5.5(c)). This indicates the silicon needles can be used to make perforate conical-shaped pores with good replication fidelity. Notably, a membrane released from a substrate seems to be freestanding out of water and its thickness is identical to the initial coating thickness, which is 5.5 µm (see Figure 5.5(d)).

In NIL, if the silicon needle contacts with a substrate without any residual layer, there will be a linear relationship between the sacrificial layer thickness and tip pore size. In other words, the tip pore diameter \(D_t\) can be expressed as

\[
D_t = 2 \left( r \left( \frac{1 - \sin \theta}{\cos \theta} \right) + l \tan \theta \right)
\]  

(5.1)

where \(r\) is the apex radius of the needles, \(l\) is the sacrificial layer thickness, and \(\theta\) is the half-

Figure 5.6 A schematic image of a conical nanopore with notations related to Eq. 5.1.
angle of the pore (see Figure 5.6). Here, three different thicknesses of sacrificial layer (100 nm, 500 nm, and 1 μm) are used and the predicted tip pore size associated with those thicknesses is 82 nm, 334 nm, and 651 nm, respectively (assume that \( r \) and \( \theta \) are 12.5 nm and 17.55°, respectively). SEM images of the tip pore patterns shows that they have an oval shape while the base pores have an octagon shape (see Figure 5.7(a)-(c)). Also, the tip pore diameter measured using SEM images is 116 ± 5 nm, 481 ± 6 nm, and 690 ± 17 nm when the thickness of sacrificial layer is 100 nm, 500 nm, and 1 μm, respectively. It indicates that the patterned pore size is larger than predicted pore size mentioned before. This happens due to the breakage of the silicon tips.
during molding, causing the apex diameter to increase. Besides, it is impossible to carry out zero residual NIL in our experiment, even if it can be possible through UV nanoimprinting with UV curable monomer solution [13] or thermal nanoimprinting with a template with high aspect ratio and thin polymer resist [14].

The deformation (i.e. the breakage) of the needles depends on the design of the stamp structure. In case of tip arrays without alignment structures, the breakage of the apex of needles after the first imprinting occurs often. Due to slightly different heights of the needles, initial contacts upon imprinting happens only for relatively tall needles, which gives rise to an increase in the pressure at the apex of needles in contact with the substrate [15]. On the other hand, for the case of one needle with alignment structures, the probability of damages to the needle was significantly reduced. Due to the self-sharpening mechanism during wet etching [12], the alignment structures are always taller than needle structures so that such increase in the pressure at the apex of needles observed for the unprotected needles does not occur. Indeed, the tip is 1.2 ± 0.1 µm shorter than the alignment structures (see Figure 5.8(a)) and it can be used several times to fabricate perforated pores without the needle deformation. SEM image of the tip pore.

Figure 5.8 SEM images: (a) the height difference between microneedle and alignment structures. (b) SU-8 residual layer beneath a cavity made by alignment structures.
patterns shows that a tip pore diameter measured using SEM images is $127 \pm 4 \text{ nm}$, $304 \pm 14 \text{ nm}$, and $375 \pm 18 \text{ nm}$ when the thickness of sacrificial layer is $100 \text{ nm}$, $500 \text{ nm}$, and $1 \mu\text{m}$, respectively (see Figure 5.7(d)-(f)). The patterned pore size with a thick sacrificial layer (>500 nm) is smaller than predicted pore size but the one fabricated with a $100 \text{ nm}$ thick sacrificial layer is larger than the expected pore size. In addition, the SU-8 residual layer was always left below alignment structures, while it was not observed below silicon needles (see Figure 5.8(b)).

For the case of one needle with alignment structures, the silicon stamp over which the needle is located might be regarded as a thin plate supported by four fixed alignment structures. The imprinting pressure should be enough in order that the plate deflection (i.e. bending) is higher than the sum of the height difference between alignment structures and needle structures and the SU-8 residual layer thickness below alignment structures. Based on the deflection of the stamp three different scenarios can happen: (1) not enough in order that the needle reaches the sacrificial layer, (2) just enough in order that the needle reaches the sacrificial layer, and (3) high enough in order that the needle reaches the substrate. The pore will be blocked, perforated but

![Schematic images of the deflection of stamp](image)

Figure 5.9  Schematic images of the deflection of stamp: (a) before imprinting, (b) not enough deflection in order that the needle reaches the sacrificial layer, (c) just enough deflection in order that the needle reaches the sacrificial layer, and (d) high enough deflection in order that the needle reaches the substrate.
smaller than the predicted value, and perforated but larger than the expected value with the breakage of the tips for the mentioned scenarios respectively (see Figure 5.9). SEM analysis shows the imprinting condition 65 °C, 1 MPa for 2 min to be suitable to form perforated pores (see Figure 5.7(d)-(f)) and the pores follow the case (3), (2), and (2), respectively. Importantly, a bur-like bump structure appears around pores and its size is proportional to the sacrificial layer thickness (see Figure 5.7). This may happen due to the SU-8 shearing and displaces the sacrificial layer along the direction of mold motion during imprinting.

5.3.3 Current-voltage measurement to determine pore size

Understanding transport properties in nanopores is crucial for building ionic circuits used for manipulation of ionic and molecular signals [16-19]. Therefore, it is interesting to perform ionic current measurements with the micro- and nanofluidic system integrated with a single pore in a SU-8 membrane (see Figure 4.4). Another advantage of performing ionic current

![Figure 5.10](image.png)

**Figure 5.10** I-V curves of nanopores fabricated on three different LOR thickness. It was imprinted with microneedles without alignments (a) and with alignments (b).
measurements is that the size of the nanopore in the membrane can be indirectly calculated from the measured I-V curves. This is particularly important since it is very difficult to measure the size of nanopores formed in polymers as the pore size becomes smaller. For the measurement, the fluidic system was filled with an electrolyte solution with known ionic conductivity. An I-V curve measurement correlated to ion transport through the nanopore was then taken (see Figure 5.10). For each membrane sample, the pore radius was calculated based on its conductance in 0.1 M KCl at ±100 mV. The slope of the I-V curve is approximately linear in this range. In theory, the tip pore radius \(r_t\) can be expressed as

\[
R = \frac{\rho L}{\pi r_t (r_t + L \tan \theta)}
\]  
(5.2)

where \(R\) is the reciprocal of the I-V curve slope, \(\rho\) is the resistivity of the electrolyte, \(L\) is the length of the pore, and \(\theta\) is the half-angle of the pore [20].

![Graphs showing nanopore size as a function of LOR thickness: without alignment (a) and with alignment (b).](image)

Figure 5.11  Nanopore size as a function of LOR thickness: without alignment (a) and with alignment (b).
Figure 5.11 shows the tip pore diameter as a function of sacrificial layer thickness. Included in the plots are pore diameters determined by SEM measurement (red line) and I-V curve measurement (blue line) with the predicted values (black line). The pore size decreases with decreasing sacrificial layer thickness. Also, the I-V curve measurement values agree fairly well with the SEM measurement values, with the averaged deviation of ±12 nm. It indicates that the electrical measurement can be used to estimate the size of the pores, in particular for polymer nanopores the size of which is hard to determine using SEM or AFM (atomic force microscopy). The polymer nanopores can melt or deform under SEM measurement due to a high-energy beam and it is not easy to use AFM due to an artifact produced by the interaction between AFM tip and sample surface.

5.4 Conclusions

In this chapter, the fabrication process developed in Chapter 3 to fabricate SU-8 membranes with micro to submicron pores was modified in order to achieve real sub-100 nm nanopore membranes, overcoming the requirements of high aspect ratio stamp fabrication and nanoimprinting. For this, silicon needles with and without alignment structures were fabricated by simple wet etching process. Both configurations successfully fabricated perforated conical nanopores with a tip diameter of about 50 nm. However, when alignment structures existed on the molds, the pore size was slightly different from each other due to the bending of silicon mold during imprinting. The difference is more noticeable when thick sacrificial layer is used and becomes negligible by decreasing sacrificial layer thickness. Nevertheless, the pore size could be successfully controlled by adjusting sacrificial layer thickness; in other words, the pore size was decreased by decreasing sacrificial layer thickness.
Also, we showed a smart route to using the SU-8 membrane as a well-defined fluid interface in a modular manner for micro- and nanofluidic system, proposing that such a membrane can potentially be used as a component for modular lab-on-a-chip devices. By using it, the feasibility of using the membrane for performing ionic current measurement in nanopores has been demonstrated. The interesting thing was the size of the nanopores in the membrane could be effectively predicted using the I-V curve.

5.5 References


Chapter 6. Voltage-Driven Transport of Ions and DNA through Conical-Shaped Polymer Nanopores

6.1 Introduction

Microneedle structures in a silicon substrate were successfully used as an imprinting mold to form conical-shaped pores with diameter less than 50 nm in Chapter 5. However, the mold structures were often damaged during thermal nanoimprint lithography (NIL). Additional alignment structures around the microneedle structures prevented the defects in the mold. As mentioned in Chapter 5, we have to consider bending of silicon molds during thermal nanoimprint lithography (NIL) when alignment structures exist on the molds. However, the focus of Chapter 5 was on controlling the pore size by adjusting the sacrificial layer thickness without an in-depth discussion of the effect of the alignment structures. In this chapter, the effect of alignment structures on the formation of conical-shaped polymer nanopores during imprinting is studied. Also, the salt dependence of ions transport through this polymer conical-shaped nanopore for KCl concentrations ranging from 1 mM to 1 M is described. By modifying the previous model [1], we show that the salt-dependent charging of our polymer nanopore explains our results at low KCl concentrations. Furthermore, we discuss DNA translocations at 0.1 M or 1 M concentration of KCl. An increase in the ionic current at 0.1 M KCl is observed, whereas a decrease in the ionic current at 1 M KCl is observed.

6.2 Experiments

6.2.1 Photomask Design

A photomask was used for patterning in a negative photoresist layer coated on a silicon wafer. The square (25 µm × 25 µm) structures positioned at an interval of 150 µm were designed
as an etching mask to form microneedle structures, including three different numbers of alignment structures (100 \(\mu\)m \(\times\) 100 \(\mu\)m). They included 0, 4, and 16 alignment structures and the distance between two alignment structures is N/A, 15,200 \(\mu\)m, and 5,000 \(\mu\)m, respectively. The etching mask is 25 \(\mu\)m \(\times\) 25 \(\mu\)m and 100 \(\mu\)m \(\times\) 100 \(\mu\)m for microneedles and alignments, respectively. Scale bar: 50 \(\mu\)m.

The fabrication of the silicon mold and conical-shaped polymer nanopores was done following the procedure described in Chapter 5. The difference is that the wet etching was performed with 40 wt% KOH solution at 60 °C and the imprinting was performed 65 °C for 2 min under various imprinting pressure with a 100 nm thick sacrificial layer.

**6.2.2 Fabrication of silicon molds and conical-shaped polymer nanopores**

The fabrication of the silicon mold and conical-shaped polymer nanopores was done following the procedure described in Chapter 5. The difference is that the wet etching was performed with 40 wt% KOH solution at 60 °C and the imprinting was performed 65 °C for 2 min under various imprinting pressure with a 100 nm thick sacrificial layer.
6.2.3 Ions and DNA transport through conical-shaped polymer nanopore

The microfluidic chip integrated with the SU-8 membrane (described in Chapter 4) used as a platform to study transport of ions and DNA through a nanopore. The chip was filled with salt and DNA solution. The solution was dropped near the inlet and withdrawn by using vacuum pump from the outlet reservoir. Additional solution was then dropped near the inlet and outlet reservoir to reduce pressure difference between them. The typical flow velocity in the microchannel is 20 μm/s [2].

The device was subsequently connected to an Axopatch 700B (Molecular Devices Corp.) patch-clamp amplifier through the reservoirs located on each side of the nanopore membrane. Current-Voltage (I-V) curves associated with ion transport through the nanopore were recorded in voltage-clamp mode using a low-pass Bessel filter with 2 kHz bandwidth. The signal was digitized with a Digidata 1322A analog-to-digital converter (Molecular Devices Corp.) at a sampling frequency of 10 kHz, and viewed with the Clampex 10.2 software (Molecular Devices Corp.). Current traces were measured at 100 kHz bandwidth using a resistive feedback amplifier and digitized at 500 kHz. Additional low-pass filtering at 10 kHz was applied.

Salt solutions were made by mixing 18.2 MΩ cm Milli-Q filtered water (Millipore Corp.) to a stock solution of 1 M potassium chloride (1 M KCl, Fluka) with 10 mM Tris (Tris(hydroxymethyl)aminomethane) and 1 mM EDTA (Ethylenediaminetetraacetic acid) (100× Tris-EDTA buffer solution, Fluka) to a pH of 8. For DNA translocation experiments, λ-DNA (500 μg/ml, New England BioLabs Inc.) was added to 0.1 M and 1 M KCl solution and the final concentration was adjusted to be 2 ng/μl. Lambda-DNA is a linear bacteriophage DNA with 48.5 kbp length (the contour length is 16.3 μm [3]) and has 12 base long single stranded overhangs that are complimentary, having sticky ends. This gives rise to concatenated molecules in free
solution and especially at high molecular concentrations. In order to prevent the concatenated molecules, the DNA solution can be heated at 50 °C for 15 min and then quenched in ice water for a minute [4].

6.3 Results and discussion

6.3.1 Fabrication of silicon mold and conical-shaped polymer nanopore

The silicon mold was fabricated following the procedure described in Chapter 5. The wet etching was performed in 40 wt% KOH solution at 60 °C for 45 min. All of needle structures formed were in a similar shape as shown in Figure 5.3. In order to check the distribution of needle heights at different locations, we measured the bottom diameters of the needles by using a microscope. The images were taken at 9 different locations as shown in Figure 6.1. After image processing (to be explained next paragraph in detail), the bottom diameters at different locations for three different samples obtained in different batches was plotted in Figure 6.2. The 3D mesh

![3D Mesh plots of microneedle bottom diameter](image)

Figure 6.2 3D Mesh plots of microneedle bottom diameter: it show that they are comparatively uniform over an entire area (8 mm × 8 mm), with an average bottom diameter of 6.1 ± 0.2 μm (a), 5.4 ± 0.4 μm (b), or 4.0 ± 0.3 μm (c), respectively. The number (indicated with blue color) represents the location in the histogram graph.
plots show that the bottom diameter is fairly uniform over the entire area. However, the diameter was slightly different at different batches, with average bottom diameter being 6.1 ± 0.2 µm, 5.4 ± 0.4 µm, or 4.0 ± 0.3 µm, respectively. As mentioned in Chapter 5, even under identical conditions, the degree of etching varies slightly so that the microneedle height should always be checked prior to using it as a mold for NIL. The needle height can be estimated from its bottom diameter using \( H = \frac{D}{2} \tan(72.45°) \), which results in 9.6 ± 0.3 µm, 8.6 ± 0.6 µm, and 6.4 ± 0.5 µm, respectively, for samples in different batches. \( H \) is the needle height and \( D \) is the bottom diameter (refer to Figure 5.3(b)).

Prior to imprinting, the mold surface was treated with a fluorinated silane in the vapor phase to reduce adhesion to the polymer resist (the molds were chosen with similar needle
height, 8.3 ± 0.1 µm, for imprinting). NIL was performed at 65 °C and 1-3 MPa for 2 min using the Obducat 6 inch commercial nanoimprinter following the procedure described in Chapter 5. After imprinting, microscope images were taken at more than 10 different locations (see Figure 6.3(a)). Using ImageJ software, the images were converted to 8-bit color from RGB (see Figure 6.3(b)) and the converted image was changed by binary inversion once more (see Figure 6.3(c)). When unexpected spots (i.e. except the spot from the base pore) appeared, these spots were extracted (see Figure 6.3(d)). By using a function for analyzing particle size, the pore size was automatically measured. The measured values were usually 10% larger than the real value (see Figure 6.3(b) and (d)). After adjusting with 10% off, the measured values were plotted.

Figure 6.4 shows the 3D mesh plots of the base pore diameter imprinted at different pressure for stamp with 0, 4 and 16 alignment structures. The results imprinted at 1 MPa pressure with different numbers of alignment structures (Figure 6.4(a), (b) and (d)) showed that the diameter was not uniform over the entire area, with the maximum deviation being 1.3 µm, 1.0 µm, and 0.7 µm, respectively, for molds with 0, 4, and 16 alignment structures. The average pore diameter was 4.7 ± 0.7 µm, 3.2 ± 0.4 µm, and 2.5 ± 0.4 µm, respectively. The pores were the largest at the center area and the pore size was decreased as the number of the alignment structures increased. Microneedles in the molds without alignment structures were broken even at a pressure under 1 MPa, while all microneedles in molds with alignment structures survived. The microneedles with 4 alignment structures were broken at 2 MPa pressure, forming an average pore diameter of 4.4 ± 0.7 µm (see Figure 6.4(c)). However, the needle with 16 alignments survived even at 3 MPa pressure. The average pore diameter at 1, 2, and 3 MPa pressure formed by molds with 16 alignment structures was 2.5 ± 0.4 µm, 2.1 ± 0.6 µm, and 2.5 ± 0.4 µm, respectively (Figure 6.4(d)-(f)). The results mean that the alignment structures help
prevent the defects of needle structures during imprinting by lowering local pressure applied at
the microneedles and thus inclusion of additional alignment structures, so that a mold can be
used multiple times for NIL.

Figure 6.4 3D Mesh plots of base pore diameter: without alignment (a), 4 alignments (b)-(c),
and 16 alignments (d)-(f). The pores were imprinted at 65 °C for 2 min under
different pressures. The number (indicated with blue color) represents the location
in the histogram graph.

Similar to estimating the mold microneedle height from the bottom diameter of imprinted
pores, the tip pore size can be estimated using $D_b = D_t + 2L\tan\theta$. $D_b$ and $D_t$ are the base and
tip pore size, respectively; $L$ is the length of membrane as about 5.5 µm; and $\theta$ is the half-
The angle of the pore as about 17.55° (refer to Eq. 5.2). The 3D mesh plots of the tip pore size imprinted at different pressure for molds with 0, 4 and 16 alignment structures are shown in Figure 6.5. Overall, the tip pore size was not uniform over the entire area. At an imprint pressure of 1 MPa, the average pore diameter was 1.2 ± 0.7 µm, -0.3 ± 0.4 µm and -0.9 ± 0.4 µm for molds with 0, 4, and 16 alignment structures, respectively (see Figure 6.5(a)-(b) and (d)). The negative sign reflects that the pore was not perforated. Plot analysis shows that the tip pore was
largest at the central area while perforation of the pore was varied depending on the number of alignment structures. The imprinting pressure is also an important factor. The tip pores were not perforated at 1 MPa when the mold with 4 alignment structures was used (Figure 6.5(b)). However, with the same number of alignment structures, the tip pores were perforated at 2 MPa with an average pore diameter of 0.9 ± 0.7 µm (Figure 6.5(c)). All of the pores patterned using the mold with 16 alignment structures were not perforated regardless of imprinting pressure, resulting in an average pore diameter of -0.9 ± 0.4 µm, -1.4 ± 0.6 µm, and -1.0 ± 0.4 µm at 1, 2, and 3 MPa, respectively (Figure 6.5(d)-(f)). The results indicate that in the design of mold structures the number of alignment structures should be chosen carefully. The number should be large enough to prevent damage to the mold structures while at the same time the alignment structures should not be too many so that the NIL process requires too much pressure to achieve perforation of the tip pore.

The tip pore size estimated by the calculation from the base pore size may be different even though they are extracted from same sample. For determination of the actual pore size, a microscopic metrology such as SEM, TEM or AFM needs to be performed. However, it is difficult to measure the tip pore size using those methods when the pore size is less than 20-30 nm, mostly due to the damage of the pore. Therefore, the pore size is indirectly determined with electrical measurements (refer to Chapter 5).

6.3.2 Ion transport through polymer nanopores for different KCl concentration

Figure 6.6(a) shows I-V curves for a conical-shaped polymer nanopore filled with different KCl concentrations ranging from 1 mM to 1 M. The tip diameter of the nanopore $D_t$ was 10.5 nm, as measured by SEM. The SEM images are shown in Figure 6.7. The base
diameter of the nanopore $D_b$ was calculated from the measured $D_t$ using $D_b = D_t + 2L\tan^\theta$ (refer to Eq. 5.2). The calculated value was $3.5 \pm 0.1 \, \mu m$, which agrees remarkably well with the average diameter of $3.2 \pm 0.4 \, \mu m$ determined via optical microscopy.

For all KCl concentrations, the I-V curves showed a linear behavior without any noticeable ion rectification behavior [5]. The slope of the I-V curve in the range of $\pm 100$ mV was used to determine the conductance, $G$, of the nanopore. The obtained conductance values were plotted as a function of KCl concentration, which is shown as red square dots in Figure 6.6(b). The black line of Figure 6.6(b) was the simulated conductance values for various KCl concentration using the measured tip pore diameter $D_t$ and Eq. 6.1:

$$G_{bulk} = \frac{\pi}{4} L (\mu_K + \mu_{Cl}) n_{KCl} e D_t D_b$$

(6.1)

where $\mu_K$ and $\mu_{Cl}$ are the ion mobility of $K^+$ and $Cl^-$, respectively; $n_{KCl}$ is the number

Figure 6.6  I-V curves of nanopore with different KCl concentration (a) and its calculated conductance (b).
density of potassium or chloride ions; and \( e \) is the elementary charge. Eq. 6.1 was derived as follows. First, the bulk conductance \( G_{bulk} \) of an electrolyte-filled tube of variable cross-section is described by [6]

\[
G_{bulk} = K \left( \frac{dz}{\pi \left( r(z)^2 \right)} \right)^{-1} \tag{6.2}
\]

where \( K \) is the electrolyte conductivity and \( r(z) \) is the radius as a function of distance along the its length (\( L \)). For a conical-shaped pore, \( r(z) \) is described by

\[
r(z) = \left( \frac{R - r_0}{L} \right) z + r_0 \tag{6.3}
\]

where \( r_0 \) and \( R \) are the radius of tip pore and base pore, respectively. Then,

\[
G_{bulk} = K \left( \frac{1}{\pi \left( \frac{R - r_0}{L} z + r_0 \right)^2} \right)^{-1} = \pi K \frac{r_0 R}{L} \tag{6.4}
\]

Consequently, the bulk conductance of an electrolyte-filled conical-shaped pore is

\[
G_{bulk} = \frac{\pi}{4 L} K D_t D_b \tag{6.5}
\]

where \( K \) can be replaced with \((\mu_K + \mu_{Cl}) n_{KCl} e\). Using Eq. 6.5 and the measured conductance values, the tip pore size was reversely calculated at different KCl concentrations. For the calculation, we used values of \( \mu_K = 7.616 \times 10^{-8} \text{ m}^2/\text{Vs}, \mu_{Cl} = 7.909 \times 10^{-8} \text{ m}^2/\text{Vs}, \) and \( e = 1.602 \times 10^{-19} \text{ coulombs} \) [1]. Also, \( n_{KCl} \) is calculated by \( 6.023 \times 10^{23} \text{ (mol}^{-1}) \times C \text{ (mol/L)} \times \)
1000 \( (L/m^3) \), where \( C \) is the KCl concentration \( (mol/L) \) \[1\]. For 1 M KCl solution, the calculated tip pore diameter was \( \sim 10 \) nm, which is in an excellent agreement with the diameter of 10.5 nm at obtained from SEM image analysis (Figure 6.7). However, significant deviation between the diameters measured by SEM and calculated from the measured conductance values was observed at low KCl concentrations. The results indicate that Eq. 6.1 or 6.5 is applicable only at high KCl concentration to determine the pore diameters (or conductance) from the measured conductance (pore diameters).

Figure 6.6(b) shows that at low KCl concentration of 1 mM to 10 mM the conductance values obtained from the measured I-V curves deviation were higher than those predicted from the measured pore diameter and Eq. 6.1. Similar behavior has been observed in nanopores and channels formed in silicon dioxide or silicon nitride membranes \[1\]. This was attributed to the variation of surface charge at the nanopore surface at different KCl concentrations. Such variation of surface charge was negligible at high salt concentration. SU-8 is an epoxy-based negative photoresist, which has a negative surface charge (\( C=O^- \) or \( COO^- \) group) when it was activated by \( O_2 \) plasma \[7, 8\]. The negative surface charge of the polymer nanopore is screened by \( K^+ \) ions in the electric double layer when the pore was immersed in a KCl solution. Upon

---

**Figure 6.7** SEM images: tip pore of conical-shaped nanopore.
applying voltage from each side of the nanopore membrane, the $K^+$ ions move towards the cathode along the double layer and as a result contribute to the overall ionic current [1]. Therefore, in addition to the bulk conductance, the conductance occurring along the electric double layer or surface conductance $G_{surface}$ needs to be considered to understand the I-V curves obtained using nanopores.

The surface conductance by counterions shielding the surface charge at the nanopore surface can be described with the following equation [1]:

$$G_{surface} = \mu \sigma \left( \int \frac{dz}{2\pi r(z)} \right)^{-1}$$

(6.6)

where $\mu$ is the mobility of the counter-ions and $\sigma$ is the surface charge density. For a conical shaped nanopore with the cross-sectional profile described using Eq. 6.3, the surface conductance is then,

$$G_{surface} = \mu \sigma \left( \int_{0}^{L} \frac{1}{2\pi \left( \frac{R-r_0}{L} z + r_0 \right) \ln (R/r_0)} dz \right)^{-1} = 2\pi \mu \sigma \frac{R-r_0}{\ln (R/r_0)}$$

(6.7)

Eq. 6.7 can be rewritten as:

$$G_{surface} = \frac{\pi}{L} \mu \sigma (D_b - D_t) \ln (D_b/D_t)$$

(6.8)

The total conductance is the sum of the bulk (Eq. 6.1) and surface (Eq. 6.8) terms and can be written as:
\[ G = \frac{\pi}{4L} \left( \mu_K + \mu_{Cl} \right) n_{KCl} eD_t D_b + \frac{\pi}{L'} \mu_K \frac{\sigma(D_b^+ - D_t)}{\ln (D_b^+/D_t)} \]  \hspace{1cm} (6.9)

The surface conductance by counter-ions is limited at the narrow end of the polymer tip pore, which is usually in the length range of about 50–200 nm from the end of the tip pore [9]. Thus \( D_b \) and \( L \) need to be replaced by \( D_b^* \) and \( L^* \), where \( L^* \) is the length in which the surface conductance applies and \( D_b^* \) is the diameter at \( L^* \). Like \( D_b \) in Eq. 6.5, \( D_b^* \) can be calculated by \( D_b^* = D_t + 2L'tan\theta \). \( D_b^* \) is 42 nm, 73 nm, 105 nm, and 137 nm when \( L^* \) is 50 nm, 100 nm, 150 nm, and 200 nm, respectively.

Eq. 6.9 describes the conductance as a function of salt concentration. The surface charge also depends on the ion concentration of the bulk solution. This is a result of the chemical reactivity of the SU-8 surface given by [7, 8]:

\[ R - CHO \rightarrow R - C = O^- + H^+ \text{ or } R - COOH \rightarrow R - COO^- + H^+ \]  \hspace{1cm} (6.10)

If the reaction is in thermodynamical equilibrium, the concentration of \( H^+ \) ions near the surface is determined by the local electrostatic potential [1]. The relationship between the potential at the diffuse layer, \( \zeta \), and the surface charge density, \( \sigma \), was described by [10]:

\[ \zeta(\sigma) = \frac{k_B T}{e} \ln \left( \frac{-\sigma}{e\Gamma + \sigma} \right) + \frac{k_B T \ln(10)}{e} (pK - pH) - \frac{\sigma}{c} \]  \hspace{1cm} (6.11)

where \( k_B T \) is the thermal energy, \( \Gamma \) is the surface concentration of chargeable sites, \( pK \) is the equilibrium constant, \( pH \) is the concentration of protons, and \( C \) is the phenomenological capacity of the Stern layer. Another relationship between \( \zeta \) and \( \sigma \) was given by [10]:

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where $\varepsilon_0$ is the permittivity of the solution and $\kappa^{-1}$ is the Debye screening length that corresponds to:

$$\sigma(\zeta) = \frac{2\varepsilon_0 k_B T \kappa}{e} \sinh \left( \frac{e\zeta}{2k_B T} \right)$$

(6.12)
Combining Eqs. 6.11 and 6.12, $\sigma$ can be obtained as a function of salt concentration (see Table 6.1). Using different $\sigma$ values at different salt concentration, the conductances were obtained from Eq. 6.9 with various $L^*$ values in the range of 50–200 nm. Other parameters $D_t$, $D_b$, $L$, $\mu_K$, and $\mu_{Cl}$ were the same as previous calculation. The blue lines in Figure 6.8 correspond to the calculated conductance in this way. The deviation between the calculated and measured conductance values was reduced but these two values do not agree well with each other, particular at low KCl concentration.

When bias voltage to drive ion flux through a nanopore is applied, a strong electric field was produced near and inside the nanopore. The electric field encourages ions to move electrophoretically through the fluid. The nanopore surface negative charged drives to an enhanced $K^+$ concentration inside the nanopore, leading to diffusive ion flow. This ion flow mechanism was dominated by ionic mobility and surface charge. Therefore, we also need to consider actual (i.e. effective) ion mobilities instead those used in the previous calculation. The

Table 6.1 Conductivity and effective ion mobility of KCl solution and surface charge density.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>1 mM KCl</th>
<th>10 mM KCl</th>
<th>0.1 M KCl</th>
<th>1 M KCl</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K$</td>
<td>0.095</td>
<td>0.302</td>
<td>1.334</td>
<td>11.060</td>
<td>$S/m$</td>
</tr>
<tr>
<td>$\mu_K$</td>
<td>$4.844 \times 10^{-7}$</td>
<td>$1.533 \times 10^{-7}$</td>
<td>$6.771 \times 10^{-8}$</td>
<td>$5.592 \times 10^{-8}$</td>
<td>$m^2/\text{Vs}$</td>
</tr>
<tr>
<td>$\mu_{Cl}$</td>
<td>$5.042 \times 10^{-7}$</td>
<td>$1.596 \times 10^{-7}$</td>
<td>$7.053 \times 10^{-8}$</td>
<td>$5.869 \times 10^{-8}$</td>
<td>$m^2/\text{Vs}$</td>
</tr>
<tr>
<td>$\sigma$</td>
<td>0.01</td>
<td>0.02</td>
<td>0.07</td>
<td>0.21</td>
<td>$\mathcal{C}/m^2$</td>
</tr>
</tbody>
</table>
ratio of effective ion mobilities between potassium and chlorine can be obtained from the transference numbers for 1 mM and 1 M KCl solution in Currie and Gordon [11]. Table 6.1 shows the calculated effective ionic mobilities with measured electrolyte conductivity. Indeed, the effective ion mobilities are different with initial values suggested before (i.e. $\mu_K$ and $\mu_{Cl}$ are $7.616 \times 10^{-8} \ m^2/\text{Vs}$ and $7.909 \times 10^{-8} \ m^2/\text{Vs}$, respectively) and showed a dependency on salt

Figure 6.9  Calculated conductances with different sensing length. The red lines were followed by Eq. 6.9 with effective ion mobilities.
concentration. Using Eq. 6.9, the conductance was again calculated using the effective ion mobilities, which is shown in Figure 6.9 (red line, $L^*$ as 50-200 nm). The calculated conductance was in a good agreement with the measured conductance for the entire KCl concentration range from 1 mM to 1 M. The variations of surface charges at the nanopore surface and ionic mobilities with salt concentration need to be considered to fully explain the experimentally measured ionic conductance behavior through nanopores over the entire salt concentration range [12]. In general, the total conductance of our nanopore is 1.6~5.5 times larger than SiO$_2$ nanopore in the KCl concentration range from 1 mM to 1 M because of large surface charge density. Our surface charge density is 7 times larger than one of SiO$_2$ nanopore [1].

6.3.3 Salt dependence of DNA translocation through polymer nanopores

After examining the ion conductance through polymer nanopores, we also investigated current changes occurring after we added $\lambda$-DNA into salt solution. Both increases and decreases in current was observed depending on salt concentration. Figure 6.10(a) shows the representative current traces at 1 M KCl solution. The current was recorded at 100 mV over time and the baseline level current of $\sim$ 6 nA was subtracted from the measured current. All of peaks were downward (i.e. decrease in current) but the magnitude of current decrease was not identical. This may be attributed to different types of translocation events such as linear, double local folded, single local folded or fully folded fragments of DNA molecules [13]. With this data, it was difficult to distinguish different translocation events for different types of peaks. However, the peak shown in Figure 6.10(a) #4 was dominant. Figure 6.10(b) shows the peak distribution extracted from the peaks in Figure 6.10(a). Fitting the distribution with a Gaussian function
yielded a current decrease of $50 \pm 14$ pA and a duration of $1.3 \pm 1.2$ ms. In contrast, all the peaks are shown upward at a low salt concentration of 0.1 M KCl solution (see Figure 6.10(c)). The baseline level current was $\sim 1$ nA which was subtracted from the measured current. When the peak distribution shown in Figure 6.10(d) was fitted with a Gaussian function, the current increase was $21 \pm 5$ pA, having $0.6 \pm 0.2$ ms as a duration time. In this case, the peak as shown in Figure 6.10(c) #1 was dominant.

As reported in [1], when DNA molecules pass through the nanopore, ionic current through a nanopore decreases at high salt concentration while it increases at low salt
concentration. This can be explained by (1) a decrease in the volume where the nanopore is occupied by the DNA backbone and (2) additional mobile counter-ions which shields the negatively charged phosphate DNA backbone and are brought into the nanopore by the DNA backbone. Considering both effects, the change in conductance, $\Delta G$, due to DNA translocation can be expressed as [1]:

$$\Delta G = \left( \mu_K^* q_{i,DNA}^* - \frac{\pi}{4} d_{DNA}^2 (\mu_K + \mu_{Cl}) n_{KCl} e \right) \frac{1}{l_s}$$  \hspace{1cm} (6.14)

where $\mu_K^*$ is the effective electrophoretic mobility of potassium ions moving along the DNA, $q_{i,DNA}^*$ is the effective charge on the DNA backbone per unit length, $d_{DNA}$ is the diameter of the DNA molecule and $l_s$ is the effective sensing length. The first and second terms in Eq. 6.14 represent that the conductance increases by increasing the mobile counterions brought in by the DNA backbone and the conductance decreases by decreasing the nanopore volume occupied by the DNA, respectively.

Figure 6.11 shows the $\Delta G$ values as a function of salt concentration. DNA translocation performed at 0.1 M KCl showed a positive $\Delta G$, whereas 1 M KCl yielded a negative $\Delta G$. Even though we have only two data point, we attempted to assume a linearity between the two data point to predict the critical KCl concentration ($C_{cr}$), where $\Delta G$ changes from positive to negative or vice versa. $C_{cr}$ was estimated to be $\sim 0.37$ M. This value was in a good agreement with the value reported in [1, 9]. From the line, we can also obtain $\mu_K^* q_{i,DNA}^*$ and $l_s$ in Eq. 6.14, for which values of $\mu_K = 7.616 \times 10^{-8}$ m$^2$/Vs and $\mu_{Cl} = 7.909 \times 10^{-8}$ m$^2$/Vs were used to calculate $l_s$. The obtained values of $\mu_K^* q_{i,DNA}^*$ and $l_s$ are $(2.09 \pm 0.07) \times 10^{-17}$ m$^2$/Ω and $72 \pm 27$ nm, respectively. Using the bulk ionic mobility $\mu_K$ of $(5.592 \times 10^{-8})$ m$^2$/Vs for $\mu_K^*$ for
simplicity, $q_{l,DNA}^*$ can be extracted as $(3.74 \pm 0.12) \times 10^{-10} \, C/m$ which corresponds to $0.79 \pm 0.02$ electron charges per base pair. This indicates a reduction in the charge per unit DNA length by $60 \pm 1\%$ in the nanopore compared to the bare charge of $2e^-$ per base pair in free salt solution.

Also, $l_*$ is in a good agreement with ionic conductance measurement results, where the effective nanopore length $l^*$ where the surface conductance is dominant is around 100 nm. The difference between the values obtained by two distinct measurements and one estimated by references (100 nm sensing length) is only approximately 28%. It means that our model (Eq. 6.9) works very well in predicting both the ionic conductance and resistive-pulse signals for salt concentration between 1 mM and 1 M.

Figure 6.11 The graph of $\Delta G$ values as a function of salt concentration. DNA translocations performed at 0.1 M KCl showed a positive $\Delta G$, whereas 1 M KCl yielded a negative $\Delta G$. 

Figure 6.11
6.4 Conclusions

In this chapter, the effect of alignment structures on the formation of conical-shaped nanopores via imprinting with molds containing microneedles was studied. The alignment structures helped prevent defects of microneedle structures in mold during imprinting by reducing the local pressure applied at the microneedle tips. As more number of alignment structures exists in mold, higher imprinting pressure was required to obtain perforated nanopores. Using Si molds with microneedle and alignment structures, perforated conical-shaped nanopores with a tip diameter as small as 10 nm were successfully fabricated by imprinting.

We also have characterized ions and DNA transport through the conical-shaped polymer nanopores. The tip and base pore diameter was estimated from the results of conductance measurements, which were in excellent agreement with the pore diameter measured by SEM and optical profilometry. At salt concentration below approximately 0.1 M, the measured conductance by I-V curves does not agree to the calculated conductance using the measured pore size. This was attributed to the variation of surface charge and ionic mobility at different salt concentration. Furthermore, DNA translocation experiments through the nanopore showed both current increase and decrease depending on the salt concentration used for experiment. From the results, the critical KCl concentration \( (C_{cr}) \) in which \( \Delta G \) changes from positive to negative was estimated to be \(~0.37\) M. This value was in a good agreement with the value reported in the literature [1, 9].

6.5 References


Chapter 7. DNA Uncoiling through Conical-Shaped Polymer Nanopores

7.1 Introduction

Conical-shaped polymer nanopores having 10 nm tip diameter by controlling alignment structures on a silicon mold have efficiently fabricated. However, the reproducibility of the nanopores were rather poor. In addition, to increase the resolution in sensing dsDNA with ~ 2 nm diameter by conductance measurements requires use of nanopores with the tip diameter much less than 10 nm. Thus, in this chapter an additional process named the polymer reflowing or pressed self-perfection (PSP) process were employed after nanoimprinting to further reduce the pore size. Starting with nanopores with less than 100 nm diameter, we have demonstrated nanopores in the SU-8 membrane down to ~5 nm, as estimated by I-V measurements. Additionally, DNA uncoiling through the conical-shaped nanopores and its dependence on the size of conical-shaped nanopores will be discussed.

7.2 Background

The polymer reflowing is a simple technique and already well known to form spherical or cylindrical lenses [1, 2]. The polymer reflowing uses the melting of islands patterned by photolithography. When the polymer patterns are melted over its glass transition temperature ($T_g$), it changes into a viscous state and its surface are pulled into a shape, which has minimum surface energy [3]. The melted polymer droplet must be then shaped, having certain physical constraint such as a critical angle, $\theta_c$ (see Figure 7.1). $\theta_c$ is regarded as Young’s Equation, $\gamma_{SL} + \gamma_{LG} \cos \theta_c = \gamma_{SG}$, where $\gamma_{SL}$, $\gamma_{LG}$, and $\gamma_{SG}$ are the surface tension between the substrate and liquid; between the liquid and the vapor; and between the substrate and the vapor,
respectively. In general, smaller $\theta_c$ need much more time to form lenses and it can be explained with a spreading factor $y_{width}$ for an initial ridge with aspect ratio, $x_{aspect}$ [4].

$$y_{width} = \frac{4\sin^2 \theta_c}{\sqrt{\frac{\pi}{180^\circ}} \theta_c - \cos \theta_c \cdot \sin \theta_c} \cdot \sqrt{x_{aspect}} = f(\theta_c) \cdot \sqrt{x_{aspect}} \tag{7.1}$$

The polymer reflowing also has been used to reduce the pattern size and line edge roughness and to remove fabrication defects [5]. Recently, the PSP process has demonstrated that pre-patterned nanopores with 120 nm diameter was effectively reduced down to 21 nm [6]. This method not only allowed for the reduction of the pore size but it also improved the critical dimension of the patterns by reducing the sidewall roughness of the patterns. The difference between the polymer reflowing and the PSP process is whether an additional force (i.e. pressure) is applied in vertical direction or not. In PSP process, a squeezing flow between the polymer and the additional substrate on top of the polymer is occurred. The squeezing flow refers to the viscos flow of a polymer melt in a sandwich of two discs and it was used to explain the flow behavior during nanoimprinting lithography (NIL). A simple model for the squeezing flow underneath the stamp protrusion is followed by the Stefan equation [7]:

Figure 7.1 A liquid droplet rests on a solid surface and is surrounded by gas.
\[
\frac{1}{h^2(t)} = \frac{1}{h_0^2} + \frac{2F}{\eta_0 L s^3} t
\]  

(7.2)

where \( h(t) \) is the film thickness underneath the stamp protrusion, \( h_0 \) is the initial film thickness, \( \eta_0 \) is the viscosity of the film, \( F \) is the imprint force, \( L \) and \( s \) are the length and width of the stamp protrusion, and \( t \) is the imprinting time. The final thickness \( h_f \) is same with \( h(t_f) \) and it gives the imprinting time:

\[
t_f = \frac{\eta_0 L s^3}{2F} \left( \frac{1}{h_f^2} - \frac{1}{h_0^2} \right)
\]

(7.3)

Equation 7.3 means that small (narrow) stamp protrusion will sink faster than large (wide) ones [8]. Figure 7.2 shows that comparison of the squeezing flow for nano- and microcavities [8]. If the nanocavity in Figure 7.2(a) is equivalent to the microcavity in Figure 7.2(b), which has the same total volume of the cavity, the imprinting time is faster in case of nanocavity, following Eq.
7.3. The fill factor should be also constant to improve flowing of the polymer and shorter imprinting time. In this sense, additional protrusions and cavities are helpful to improve imprinting results (see Figure 7.2(c)).

7.3 Experiments

7.3.1 Fabrication of SU-8 membrane having conical-shaped nanopore

In the PSP process (see Figure 7.3), pre-patterns in a double resist layer (SU-8 as active membrane layer and 1 µm thickness LOR as sacrificial layer) were initially produced following the procedure described in Chapter 3. However, prior to the UV exposure step and before curing of SU-8, the pre-patterned SU-8 was pressed with a transparent, flat quartz blank wafer coated with a fluorinated silane at various pressures and times. Subsequently, the SU-8 was exposed to
UV light for 10 sec, followed by baking process at 95 °C for 5 min. Finally, the freestanding SU-8 membrane was achieved by dissolving LOR layer with a MF319 solution.

7.3.2 Ions and DNA transport through conical-shaped polymer nanopores

The processes to form the microfluidic chip integrated with the SU-8 membrane and to study transport of ions and DNA through conical-shaped nanopores followed the procedure explained in Chapter 4 and 6, respectively.

7.4 Results and discussion

7.4.1 Size reduction by PSP from micro to sub-micropore

Before we applied the PSP process to reduce the nanopore size from sub-micron to nanoscales, preliminary experiments of reducing the pore size from microscale pores were performed. Figure 7.4 shows the results after employing PSP process to a membrane with 3.0 µm square wells at a processing temperature of 55 °C for 10 min with different pressures. Before PSP process, the pore was a square shape with 3.0 µm width (see Figure 7.4(a)), having identical shape and size to the silicon stamp (see Figure 3.5(f)). By applying pressure, the pore size was reduced to ~1.9 µm, 417 nm, and 325 nm at 1, 3, and 5 MPa pressure, respectively (see Figure 7.4(b)-(d)). In addition, the shape of the pores becomes circular and line edge roughness was improved. Figure 7.4(e) shows a plot of the pore diameter in polymer membrane as a function of pressing time and pressure. Since it is expected that the sidewall profile of the perforated pores after PSP process may deviate from the vertical sidewall profile of the pillars in the silicon stamp, it is difficult to define a single pore diameter. For simplicity, we just used the average diameter taken for inner dark circles in the SEM images and defined it as equivalent circular diameter. In
general, the pore diameter becomes smaller with the process time, indicating that the pore size can be easily reduced by controlling process time and pressure.

Figure 7.4 SEM images of polymer membrane on a silicon substrate after PSP process at 55 °C for 10 min: (a) before, (b) 1 MPa, (c) 3 MPa, and (d) 5 MPa. (e) shows final pore size as a function of process time (with fixed 55 °C temperature). The initial pore is 3.0 µm diameter, 15 µm period, and 4.0 µm thickness.
Figure 7.5 Cross sectional SEM images of micropore and trench before PSP ((a) and (c)) and after PSP at 55 °C, 3 MPa for 10 min ((b) and (d)).

Figure 7.5 show cross sectional SEM images before and after PSP process. The data indicate that the SU-8 flows most in A and least in B (see Figure 7.5(b)). It seems likely that the residual layer is compressed during the process, resulting in a convex profile (data not shown). Then, the polymer in the interface (A and C in Figure 7.5(b)) undergoes a higher shear than the intermediate region (B in Figure 7.5(b)) and therefore has a lower viscosity during flow. It is expected that the pore bottom (A in Figure 7.5(b)) is smaller than the pore top (C in Figure 7.5(b)) because less shear occurs in C due to silane molecule in the substrate. However, the pore bottom can be blocked due to the residual layer so that etching process should be required to remove it in order to obtain perforated pores. Another interesting thing is that the flow behavior during PSP process is different with different shaped pre-patterns. The SU-8 flows in the trench structures (C...
in Figure 7.5(c)-(d)) less than in the pore structures (C in Figures 7.5(a)-(b)). It seems likely that the uniaxial shear occurs in the trench structures while the biaxial shear causes flow around the pore structures. Consequently, above results indicate that the PSP process with pores of initially vertical sidewalls can be used to produce conical-shaped polymer nanopores.

**7.4.2 Size reduction by PSP from sub-micro to nanopore**

Similar to the size reduction from micro to sub-micropore shown in the previous section, the sub-micrometer pores (~670 nm tip pore diameter) were decreased down to ~30 nm diameter. For this, a conical-shaped nanopore was pre-patterned by using a silicon stamp having microneedles and no alignment structures (refer to Chapter 5). Figure 7.6(a)-(d) shows the SEM images taken from the base pore side after employing PSP process at a processing temperature of 55 °C for 5 min with different pressures. The initial tip pore size was 670 nm (Figure 5.7(c)). Prior to the PSP process, the base pore was in an octagon shape with 3.6 µm diameter (see Figure 7.6(a)), which matches well with the structures in the silicon stamp (Figure 5.3). By applying pressure, the base pore size decreased with increasing pressure. The base pore shape was also changed from octagonal to circular (Figure 7.6(b)-(d)). We used the measured current-voltage (I-V) curves at 0.1 M salt concentration to calculate the tip pore sizes using Eq. 5.2 (refer to Chapter 5). Figure 7.6(e) shows the tip pore diameter in the SU-8 membrane as a function of pressure for PSP. By applying pressure, the pore size was reduced to about 150 nm, 80 nm, and 30 nm at 1, 2, and 3 MPa pressure, respectively.

According to the results in the previous section, the flow behavior of the SU-8 in conical-shaped pores after PSP process can be estimated. Figure 7.7(a)-(b) show cross-sectional SEM images before and after PSP process with a conical sub-micron pore. Overall the conical
shape of the pore remained but the actual sidewall profile slightly changed in such a way that two different sidewall angles exists near tip and base pores. The sidewall angle near the base

shape of the pore remained but the actual sidewall profile slightly changed in such a way that two different sidewall angles exists near tip and base pores. The sidewall angle near the base
pore remained to be similar to that of the initial pore of 72.45°. However, the sidewall angle near the tip pore increased to 79.45°. Further experiments should be carried out to accurately explain the polymer flow behavior during the PSP process. However, the sidewall profile follows the flow schematically described in Figure 7.7(c). This is because the polymer at the interface undergoes larger shear than the intermediate region, like Figure 7.5. The tip pore was not blocked by the PSP process, which indicates that the PSP process is feasible as pore reduction process once perforation through the SU-8 layer is achieved during NIL. If the pore is not perforated during NIL, an additional plasma etching process should be performed to remove the residual SU-8 layer prior to applying the PSP process.

Figure 7.7 Cross sectional SEM images of conical-shaped pores before (a) and after PSP at 55 °C, 1 MPa for 5 min. (c) shows the estimated profile of conical-shaped nanopore during PSP.
Another interesting observation is that the flowing of SU-8 during PSP process was totally different when conical-shaped nanopores were pre-patterned by NIL using silicon microneedles with alignment structures (refer to Chapter 5). After the PSP process at 55 °C with 1-3 MPa pressure for 5 min, most pores were blocked (data not shown) because in the flow of SU-8 alignment cavities was better and the shorter embossing times. In order to slow down the flow of SU-8 during the pore reduction process, we developed the modified process named as reflowing process (PSP process without pressing) where the pre-patterned SU-8 membrane is left on a hot plate at an elevated temperature without applying pressure by a blank quartz wafer. Figure 7.8 show the SEM images before and after reflowing process for 1 min at different temperatures. For this experiment, conical-shaped nanopores with alignment structures were pre-patterned in the SU-8 layer, which was followed by the reflow process. The samples were then copied into a polyurethane acrylate (PUA) layer (MINS-511RM, Minuta Technology Co.) coated on a polycarbonate sheet. By increasing temperature, the puddle (or unfilled area) seems to be filled easily even though the processing time is really short. It indicates that the size reduction within a couple of nanometers occurs exceedingly fast and it is not easy to control. Nevertheless, the reflow process is still a simple method without using any expensive tools.

Figure 7.8 SEM image of UV resin structures after copying from imprinted structure: before reflowing (a), after reflowing at 45 °C (b), and 55 °C for 1 min (c).
7.4.3 DNA uncoiling through conical-shaped polymer nanopores

The conical-shaped polymer nanopores were pre-patterned in the SU-8 layer coated on 100 nm thick sacrificial LOR layer. The reflow process was carried out at 55 °C for 1 min. In order to obtain the tip pore size, I-V measurements were performed for different ion concentrations. The calculated tip pore diameter is 10 nm (a) and 5 nm (c).

Figure 7.9 I-V curves of nanopore with different KCl concentration (a) & (c) and its calculated conductance (b) & (d). The calculated tip pore diameter is 10 nm (a) and 5 nm (c).
concentrations. Figure 7.9(a) and (c) shows I-V curves for two different conical-shaped polymer nanopores filled with different KCl concentrations ranging from 1 mM to 1M. For all KCl concentrations, no ionic rectification behavior was observed, showing only linear curves. The slope of the I-V curve in the range of ±100 mV was used to determine the conductance, \( G \). The obtained conductance values were plotted as red square dots as a function of KCl concentration (Figure 7.9(b) and (d)). Using the method described in Chapter 6, the tip pore size for the two samples after the reflow process were obtained, which resulted in 10 nm and 5 nm, respectively.

Prior to adding DNA solution to the microchannel, the current trace was recorded to obtain baseline \( (I_0) \) for 1 M KCl solution. After adding 1 M KCl solution contained with 2 ng/\( \mu \)l \( \lambda \)-DNA, we let sufficient time elapsed in order to minimize the effect of bulk flow moving in the microchannel. The bulk flow in the microchannel usually occurs due to the pressure difference present between two reservoirs or to the inertia occurring when the buffer was introduced (e.g. injection or withdrawal). Typically, The bulk flow velocity in the microchannel is 20 \( \mu \)m/s or more, which may affects the capture of DNA into nanopores [9]. Within a few minutes after 100 mV was applied, many current peaks were observed as shown in Figure 7.10 (a) and (c). Both current traces looked similar to the previously reported data in the literature which were recorded using protein or solid-state nanopores [10, 11]. The frequency of the peaks (the capture frequency \( (f) \)) increased with increasing with pore diameter, with \( f \) being 138 ± 12 min\(^{-1}\) and 498 ± 62 min\(^{-1}\) for the 5 nm and 10 nm pores, respectively. In addition, only peaks with small magnitude were observed for the 5 nm pore while peaks with a wide range of magnitudes appeared for the 10 nm pore.

The capture frequency \( f \) of DNA into a nanopore can be understood with the capture mechanism. The capture mechanism consists of two main steps: the first step is the transport of
DNA in the microchannel bulk flow which is limited by diffusion (diffusion-limited regime) and the second step is the transport in the vicinity of the nanopore entrance which is limited by an entropic barrier (barrier-limited regime). For DNA molecules with the molecular weight between 8,000-48,000 bp, the transport is mostly limited by diffusion in the microchannel while the transport is mainly limited by entropic barrier for DNA molecules with the molecule weight rage ~800-8,000 bp [12]. In the diffusion-limited regime (in the first step), a DNA coil moves to the pore from bulk to a distance larger than the DNA coil size, $r_g$. At that time, its motion follows a biased path, defined by the electric field outside the pore, instead of purely diffusive [12]. This

Figure 7.10 The current traces of 10 nm (a) and 5 nm pore (b). It was measured with 1 M KCl. (b) and (d) show the peak distribution of (a) and (c), respectively.
field exists because KCl solution is an ionic conductor, not a dielectric so that it is not subject to Debye screening [13]. As a consequence, this field is maintained by an ionic current across the pore that generates a potential profile $V(r)$ outside the pore mouth from a distance $r$ [12]. The DNA coil attracted by this field then moves to the pore mouth. In barrier-limited regime (in the second step), when the DNA coil closes to within nearly one coil size of the pore ($r_g$), a DNA end moves into the pore to overcome free-energy barrier.

In this study, $\lambda$-DNA with a molecular weight of 48.5 kbp was used. Therefore, the DNA capture was dominated by transport diffusion-limited regime. The capture rate in the diffusion-limited regime ($R_{diff}$) is given by [12]:

$$R_{diff} = \frac{\pi d^2 \mu}{4l} \Delta V$$

(7.4)

where $d$ is the diameter of the nanopore, $l$ is the length of the nanopore, $\mu$ is the DNA free solution electrophoresis mobility, and $\Delta V$ is the voltage applied to the electrodes. Based on Eq. 7.4, $f$ for 10 nm pore diameter should be four times larger than that for the 5 nm pore diameter, which agrees well with our results. Our pore is not in a cylindrical shape which was assumed in Eq. 7.4 [12]. The difference in the pore shape will mostly influence the electric field in the pore but the relative electric field in the microchannel and pore entrance areas for different pore sizes is not expected to vary significantly. Thus, Eq. 7.4 is still applicable to the determination of relative capture rates for different pore sizes.

High resolution I-t curves provide detailed insight on the conformational behavior of the DNA molecules during translocation through nanopores (see Figure 7.10(a) and (c)). The shape of the peaks obtained for different pore sizes is significantly different. For a 10 nm pore, most peaks were sharp with a small degree of asymmetry. Peak #4 occurred most frequently while the
peak #3 occurred sporadically (Figure 7.10(a)). For the 5 nm pore, various peak shapes appeared. As shown in peak #1 of Figure 7.10(c), single events with having multiple levels of current occurred. Peak #3 appeared most frequently while the peak #1 rarely occurred. In order to compare the behavior for different pore sizes, the time duration of the translocation and the blockade current for each event were measured. The width of the peak on the baseline was used as the time duration of the translocation and the peak amplitude corresponds to the blockade current.

Figure 7.10(b) and (d) show typical statistics of the DNA translocation events for the two nanopore sizes. Fitting of the distribution with a Gaussian function yielded the peak amplitude and duration of $23 \pm 7$ pA and $10.4 \pm 2.6$ ms, respectively, for 5 nm pore and $50 \pm 14$ pA and $1.3 \pm 1.2$ ms for 10 nm pore. In general, the blocking current increased with increasing pore size but the duration decreased with the larger pore size. A shorter duration time also reflects a higher blockade current. Such a relationship indicates that not only individual DNA chain remains in its less stretched state during its translocation but also its stretching (i.e. uncoiling) significantly depends on pore diameter. Each DNA chain can pass through a large pore without a change of its coiled conformation, while individual DNA chain must be stretched and pulled through a smaller pore.

When each DNA chain is stretched, its first segment entering the pore will generate tension on the rest of the segments still outside the pore. If the relaxation time ($\tau_R$) of the rest of the segments outside the pore is not enough because of a higher applied voltage, the rest segments are engaged with each other and trapped outside of the pore entrance (see Figure 7.11). Consequently, complicated current profiles appear (see Figure 7.10(c)). When the stretching time ($\tau_s$) of the first segment inserted into the pore was much smaller than $\tau_R$. In this experiment, $\tau_s$
Figure 7.11 Shematic of translocation dynamics of dsDNA chain through a conical-shaped polymer nanopore. (a) step A: a DNA chain electrical driven moves towards the entrance of the pore, decreasing current, (b) step B: the segment inside the pore is strecthed by the electric force, increasing current slightly (c) step C: the rest of the chain outside the pore stucks when the relaxation time is not enough at high applied voltage, decreasing current shortly, and (d) step D: the interlocked chain segments outside the pore is gradually released, increasing current.

Estimated from Step B in Figure 7.11 was 0.84 ± 0.19 ms at 100 mV, while $\tau_R$ was about 7.2 ms. The relaxation time ($\tau_R$) was estimated by the relaxation time of the first Rouse-Zimm normal mode for a DNA chain [14],

$$\tau_R = \frac{1}{(3\pi)^{1/2}} \frac{\eta_s b^3 N^{3/2}}{k_BT} = \frac{1}{(3\pi)^{1/2}} \frac{\eta_s R_F^3}{k_BT}$$ (7.5)

where $b$ is the Kuhn length, $N$ is the number of Kuhn segments per chain, $\eta_s$ is the solvent viscosity, and $R_F$ is the average chain end-to-end distance. For ideal chain, $R_F^2 = b^2 N$ and $R_G^2 = b^2 N/6$, where $R_G$ is 0.88 µm and $\eta_s$ is 904.1 µPa·s. These current profiles are similar to previous data recorded with conical glass pores (14 nm pore diameter), which have 0.48 ± 0.16
ms as $\tau_s$ at 300 mV [14]. Although the applied voltage we used is lower, DNA uncoiling was still detected. An electric field near the pores increased by decreasing pore diameter. Also, our longer $\tau_s$ seems to be due to the lower applied voltage.

7.5 Conclusions

In this chapter, we have characterized the PSP process and reflow process which was used to reduce the pore size from micrometer to sub-micrometer scales and from sub-micrometer to nanometer scales. Using the reflow process which was performed at an elevated temperature without applying pressure, a conical-shaped polymer nanopore as small as 5 nm in the tip diameter was achieved. We performed DNA translocation experiments with 5 and 10 nm pores fabricated by the reflow process and characterized the current versus time curves obtained at 100 mV. Peaks were mostly sharp for the 10 nm pore and each peak had a single level current. With the 5 nm pore, on the other hand, peaks with different shapes occurred. The results were explained by different conformational behavior of the DNA molecular chains in and in the vicinity of the nanopore.

7.5 References


Chapter 8. Conclusions and Future Work

8.1 Conclusions

The main goals of this research was to develop a process, allowing low-cost and high-throughput fabrication of freestanding polymer membranes having perforated micro- and nanopores, and to integrate the membranes into polymer microchannels. The use of 3D micro- and nanofluidic devices for ions and DNA transport was studied. A few preliminary steps were accomplished: (1) designed and fabricated high quality silicon molds for nanoimprinting lithography (NIL), (2) developed the NIL process to achieve freestanding polymer membranes having micro- and nanopores, (3) developed an integration process to assemble freestanding membranes into microchannels to build 3D micro- and nanofluidic devices, and (4) used this 3D micro- and nanofluidic devices to study the ions and DNA transport behavior through the pores.

First, the suitable imprinting molds were designed and fabricated to make perforated micro- and nanopore in polymer membranes using conventional photolithography and etching processes. Microneedle structures were chosen as the mold to fabricate conical-shaped nanopores because they are extremely high aspect ratio structures so that it is useful to achieve high mechanical stability for the membrane fully released from a substrate. Alignment structures were added to the mold to prevent breaking of needle structures.

Established NIL was with the addition of sacrificial layer techniques to make freestanding polymer membranes. The pore diameter could be control by adjusting the thickness of sacrificial layers, by using molds with different alignment structures, and by using polymer reflow. Through these methods, less than 10 nm pore diameter in freestanding polymer membranes were reliably, repeatedly produced. These membranes could be easily integrated into polymer microchannels so that 3D micro- and nanofluidic devices were realized.
The 3D micro- and nanofluidic devices were used to characterize ionic and DNA transport through the conical-shaped polymer nanopores. At KCl concentration below approximately 0.1 M, the conductance did not follow the bulk conductance because of surface charge effects. Furthermore, the translocation of DNA caused an increase in current at KCl concentrations below approximately 0.37 M, while the translocation of DNA produced a decrease in current at concentrations higher than 0.37 M. DNA uncoiling was only observed in 5 nm pore, having multiple levels of current peaks.

Figure 8.1  SEM images of microneedles after wet etching at 80 °C in 35 wt% KOH solution without isopropyl alcohol (IPA). From (a) to (c) is center to edge of sample.

Figure 8.2  SEM images of microneedles before (a) and after imprinting at 65 °C, 5 MPa for 2 min (b-c).
8.2 Future work

8.2.1 Developing disposable imprinting stamp

As mentioned in Chapter 5, the silicon etch rate can be different and different planes of microneedles can be developed during wet etching. Figure 8.1 shows typical SEM images to demonstrate this problem. The data indicate that the etch rate at the center area is faster than at the edge because ions from the KOH solution dwell longer at the center than at the edge. Even though this problem can be prevented by controlling the stirring speed and using a new KOH bath, the fabrication of microneedles is difficult and time-consuming. Also, the silicon stamp can be broken after using several times for imprinting even though there are alignment structures, as depicted in Figure 8.3.

![SEM images of UV resin stamp before (a, c) and after imprinting 65 °C, 5 MPa for 2 min (b, d). (a, b) is copied from silicon master and (c, d) is copied from PDMS master that was replicated from silicon microneedles.](image-url)
shown in Figure 8.2. Therefore, the silicon stamp should be used for replicating with other materials that are easier to handle and less expensive.

One of the examples is UV resin [1]. Figure 8.3 shows SEM images of UV resin stamp after copying from silicon master and PDMS replica. The data shows that UV resin stamps can be easily copied but their apex sharpness is different with different master materials. The apex (as shown in Figure 8.3(a)) is sharp when it was directly copied from a silicon master. However, the apex (as shown in Figure 8.3(c)) is not sharp when it was copied from a PDMS replica. It seems likely that the surface of PDMS replica with narrow cavity (i.e. microneedle shape) is hydrophobic so that UV resin cannot reach to the apex. Although the UV resin stamp can be easily replicated, the mechanical strength is not enough to use as an imprinting mold, as shown in Figure 8.3(b) and (d).

8.2.2 Studying ions and molecules through nanopores

In natural systems, the cell or plasma membrane separates the interior of all cells from the environment. Cell membranes, selectively permeable to ions and organic molecules, control the movement of substances in and out of the cells through ion channels. The ionic transport properties of ion channels are mainly composed of three characteristic features including ionic selectivity, ionic rectification and ionic gating. They depend on different external stimuli such as the pH, temperature, light, electric potential, ions, and molecules. Chemical modification with functional molecules enables to synthetic nanopores to respond to those stimuli, behaving like natural ion channels.

Among the stimuli, pH responsive synthetic nanopores have been extensively studied. Since pH is the most important change in natural systems and is a significant factor for
electrochemical reactions. When polyethylene terephthalate (PET) nanopores were prepared with a tack-etching technique, its surface was negatively charged, having asymmetric shape (conical shape) [2]. At high electrolyte pH (< the isoelectric point of the polymer surface), the surface will be negatively charged. However, at low electrolyte pH, the surface will be positively charged due to protonation of the surfaces and the adsorption of protons, eliminating the ion rectification properties. Ion rectification is pH sensitive and many other materials also have similar properties [3]. In this regard, SU-8 membranes with nanopores prepared by NIL are expected to have similar properties due to an O₂ plasma process. Meanwhile, the ion rectification phenomenon was controlled by chemical modification with amino groups [3, 4], motor DNA molecules [5], and the amphoteric molecules or pH-responsive polymers [6-8].

Polymer nanopores have been used sensing to characterize analytes. Harrell and co-workers discriminated between single-stranded DNA (ssDNA) and double-stranded DNA (dsDNA). They tuned the diameter of polycarbonate (PS) pores to allow the selective passage of ssDNA but rejection of dsDNA [9]. Mara and co-workers discriminated between molecules (dsDNA) of different lengths and shapes [10]. Sexton and co-workers used gold-plated PET pore to discriminate larger complexes from individual BSA and antibody proteins [11]. Siwy and co-workers also used gold-plated PET pore to bind ricin analyte. It leads to permanent and complete pore blockade [12]. Heins and co-workers detected porphyrin through nanopore as a function of applied voltage and porphyrin concentration [13]. Therefore, SU-8 nanopores can be expected to be suitable for use in sensing and characterizing various analytes such as DNA and RNA; peptides, proteins, and protein complexes; and small molecule chemistry.
8.3 References


Vita

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