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Research paper

Separation of Biological Molecules using Electrochemically Etched Nanoporous Silicon Membrane

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Abstract

This paper presents a separating technique of biological molecules and non-biological particles in solution using nanoporous silicon membranes. The simple separation method has been studied by fabricating the porous silicon membrane with Polydimethylsiloxane (PDMS) to produce the filtration system. The testing procedure began with diluted and sonicated indium oxide particles with deionized (DI) water and mixed with a biological solution. In this experiment setup, biological particles used is protein standard, serum albumin and sodium chloride has been filtered out through this filtration system. As a result, the indium oxide particles were trapped on the nanoporous silicon surface. Meanwhile, biology molecules penetrate the nanoporous silicon membrane with particle and molecule sizes up to 15 nm. The filtered-out particles are inspected under Zetasizer Nano SP to count and measure the size of the particles and molecules. According to the inspected result show 98% biological molecules are filtered out from this nanoporous silicon membrane. Due to this simple fabrication process, porous silicon membranes are able to be integrated to the other component to develop the complete bioMEMS and Lab on Chip system in the future.

Keywords: *Nanoporous silicon membrane, PDMS, electrochemical etching, biological molecules, indium oxide*

1. Introduction

Separating particles for biological solution or molecules are widely used in bioMEMS or lab on chip application. The separating particles can be done using the porous membrane, micropillar [1], microchannel and dielectrophoresis [2, 3]. The porous silicon and micropillar use the same concept of filtration using the mechanical behavior like external pressure to separating the particles. But for electrophoresis used non-uniform electrical field to separating the particles. The techniques of separating are different based on application. For example, separating micro sized particle likes virus, cell and bacteria commonly used the micropillar or dielectrophoresis for separating techniques. But for separating biological molecules like protein, albumin, ions used a nanopores. Nanopores is widely used especially for artificial kidney application for filtering the solute in human blood [4]. Table 1 shows the size of various components in human blood.

Table 1: Typical sizes of various components in human blood [1]

Biological component / contaminant	Typical sizes
Globular proteins (enzymes, hemoglobin, immunoglobulins, albumins)	3–20 nm
Viruses (influenza A, HIV)	100–120 nm
Small bacteria (mycoplasma)	150–250 nm
Large bacteria (E.coli)	1–2 µm
Red blood cells (erythrocytes)	7–9 µm
White blood cells (leukocytes)	7–15 µm

There are several techniques to produce nanoporous silicon which is anodization or electrochemical etching process, rapid thermal annealing, ion track etching, focus ion beam or electron beam lithography in order to produce the structure of channel. According to this fabrication technique, electrochemical etching and rapid thermal annealing is very difficult to get uniform pore in terms of distribution and size. Characterization procedure is performed to get the finest pore structure. These fabrication process enquired characterization process by manipulate various parameter to get desired pore structure, size and good distribution. Electrochemical etching techniques are very simple fabrication process yet economical process compared to the other aforementioned techniques. For ion track etching, focus ion beam and electron beam lithography is the most common techniques used in order to get the very uniform size of channel structure by customizing the size and structure. However, the simplest experiment setup and the lowest cost of fabrication will give an advantage in this process.

The finest tune of producing nanoporous silicon has been characterized and the efficiency of porous silicon to separate or filter particles has been tested [2, 3]. The complete filtration system used by attached or bond the nanoporous silicon with polymer likes Polyurethane Methacrylate (PUMA) [4] and Polydimethylsiloxane (PDMS) [5] for testing the functionality to filter out the wanted and unwanted particles.

In this paper, the pores are formed on a silicon membrane to separate biological molecules such as protein standard, human serum albumin and sodium chloride using electrochemical etching techniques. In the early stage of experiment, the size of indium oxide, protein, albumin and sodium chloride are inspected and measured using the *Zetasizer Nano SP* as a reference value for testing procedure. The Zetasizer measures the molecular weight and the size of particles based on the light scattering and converts it to particle counts based on the size or the volume.

For the testing procedure, indium oxide particles are mixed with the biological solutions separately. Indium oxide particles are very stable particles and it will be difficult to contaminate the biological molecules [6]. The particles that pass through the nanoporous membrane will be inspected under the Zetasizer Nano SP to count the particles sizes in the solution. This testing procedure is repeated for the mixture of Indium oxide with serum albumin and sodium chloride. At the end of the process, it is expected that the biological molecules are successfully filtered out through the nanoporous silicon membrane. This filtration system can be applied to the artificial kidney application especially on separating small particles like protein, albumin and ions to mimic the filtration system inside the human kidney.

This paper presents separation of biological molecules using electrochemically etched nanoporous silicon membrane. In this study, nanoporous silicon membrane is used as filtration medium to separate the biological particles from non-biological particles. The optimum process to produce 20-30 nm pores has been study and discussed in previous research paper. In this paper will highlight the simple and easier method of filtration system without using optical micrograph or extra preparation to study the capabilities of nanoporous silicon to separate biological particles. From this experiment setup, 90% of biological particles has been filtered out by analyzing and extract the data from Zetasizer Nano SP. This method capable to get good result as experiment setup using optical micrograph.

2. Fabrication Process

The membranes were began fabricated on <100> 400 µm thick doubled-sided polished (DSP) silicon and pre-coated on both sides with 200 nm thick of silicon nitride and undergo the lithography process to pattern square frames on the substrate. The square pattern (2 mm x 2 mm) has been transferred on silicon nitride substrates. Next, the samples were dipped into a buffer oxide etch (BOE) solution to remove unwanted nitrides at the frame openings. This solution is used to etch thin films of silicon dioxide (SiO_2) or silicon nitride (Si_3N_4) especially in a microfabrication process. Next, the samples were dipped into a 45% Potassium Hydroxide (KOH) + 10% Isopropyl Alcohol (IPA) solution with a constant temperature of 75 °C for 7 hours to thin the bulk silicon until it reached approximately 5 µm thickness. A silicon membrane with thickness 4.58 µm is formed under the aforementioned parameters for 6 hours 30 minutes immersion.

After the silicon membrane get the desired thickness, the silicon membrane was dipped again in BOE solution for 10 minutes under 80 °C temperature to remove all nitride layer. The nitride layer will affect the electrochemical etching (ECE) process during producing the pores. If the nitride layer were trace in silicon membrane, ECE process were not formed the pores on silicon membrane surface. In this case, the ECE process will remove the nitride layer. So, this condition will occur contamination issues in electrolyte solution for an electrochemical etching (ECE) process. Porous silicon can be formed using electrochemical etching or anodization process using hydrofluoric acid (HF) solution as the base material [7-10]. Commonly, porous silicon cannot be formed by dipping in HF solution only. The current flow between two electrodes will produce the pore on silicon membrane. The current between two electrodes was measure by current supply per area of silicon which called as current density. The basic process of ECE setup was dipping the two electrodes which are silicon and platinum wires and immersed in an electrolyte solution containing 49% HF.

During ECE process, there are several parameters that has been studied in controlling nanopores formation. The method used is called the self-adjusting method and depends on the dopant, current density, HF concentration, diluent, light, electrolyte stirring, orientation, temperature and etching time. These parameters will affect the pore formation especially on the shape, size, structure and uniformity of the pores on the silicon membrane. The ECE process was repeated process to get the desired pore formation in terms of size and structure. Field Emission SEM (FESEM) was used to inspect and verify whether the pore structure formed was suitable in order to build the filtration system. There are certain requirements that need to be met in order to separate the particles efficiently. The first requirement was to get the columnar pore structure. The second was to get the pore size to be less than 50 nm. In the end of characterization process, the pore with size 20-30 nm has been produced using this experiment setup. Based on the aforementioned parameter which was volume ratio 5:5 between ethanol and hydrofluoric acid and high current density 600mA/cm^2, the range of pore produced is almost same with difference size of ± 5 nm.

After the desired pore structure and size was formed, this nanoporous silicon membrane will be bonded with PDMS to attach silicon and tube for the testing procedure. The tube was used as the inlet and outlet to interconnect the PDMS and nanoporous silicon membrane. The bonding fabrication process between the PDMS and nanoporous silicon membrane is used to ensure that the particle solution will not leak during testing.

PDMS is used to bond the tube and silicon membrane. Firstly, 1 mm holes are drilled in the petri dish to place the tygon tube on the petri dish. After that, PDMS is prepared under room temperature by measuring the its weight and curing agent by a 10:1 ratio. Then, PDMS is stirred with the curing agent until PDMS forming bubles. So, the solution needs to be put inside vacuum chamber for 30 minutes to remove the bubbles through PDMS surface oxidation. After that, the PDMS is poured in the petri dish and cured in the oven at 50 – 60 °C for 1 hour. Then, the tube with PDMS will be cut to 1.5 x 1.5 cm and attached with the nanoporous silicon membrane. Fig. 1 shows the fabrication process to prepare and bond PDMS with nanoporous silicon membrane, as shown in a) the condition of PDMS and curing agent after being stirred in petri dish, b) pouring of bubbleless PDMS in petri dish to glue tubing, c) solid PDMS peeled off from petri dish and cut to 1.5 x 1.5 cm d) the bonding of the tubing with the nanoporous silicon membrane. The cross section of complete filtration system as depicted in Fig. 2.

The Zetasizer Nano SP able to count particle sizes from 0.3 nm up to 10 µm. For the molecules of size 0.3 – 15 nm, they can normally be measured by molecular weight. The Zetasizer Nano will convert the molecular weight to the size of particles. The size of protein standard solution (Sigma Aldrich 80mg/mL) and serum albumin (Sigma Aldrich protease free) can be inspected under the Zetasizer Nano to examine their molecular weights or the size of particles in the biological solution. The size of protein standard is found to be 4 nm to 11 nm while the serum albumin measures 3 nm to 8 nm and sodium chloride is 0.5 nm to 2 nm.

In this testing procedure, the new sample was used for different mixture of biological and non-biological particles and molecules. The first experiment starts with the mixture of indium oxide and protein standard, indium oxide and serum albumin. For the last experiment setup, the mixture of indium oxide and sodium chloride was used to observed the working filtration membrane.

For testing preparation, Indium oxide was diluted in DI and sonicated in ultrasonic bath before mix with the biological particles to avoid agglomeration. Then, the syringe pump is connected at the inlet and outlet of the filtration system for testing. A 0.3 mL solution is in-

jected gently in the tubing with a flow rate of 100 µL/min for 3 minutes. This flow rate is used to mimic the kidney filtration system in filtration system.

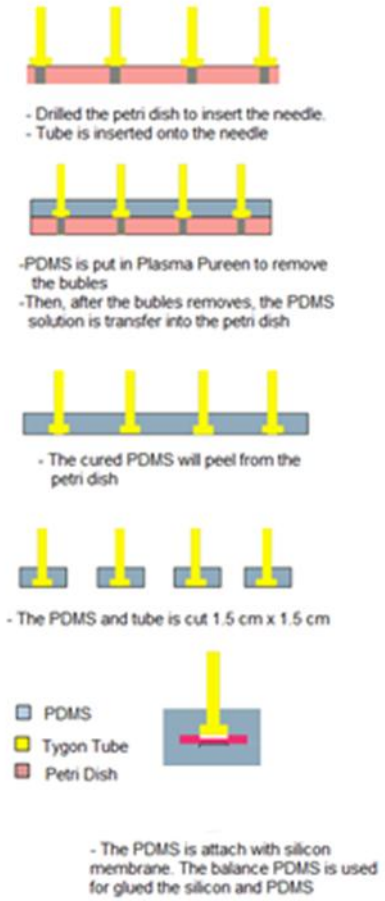

Fig. 1: PDMS preparation for filtration system

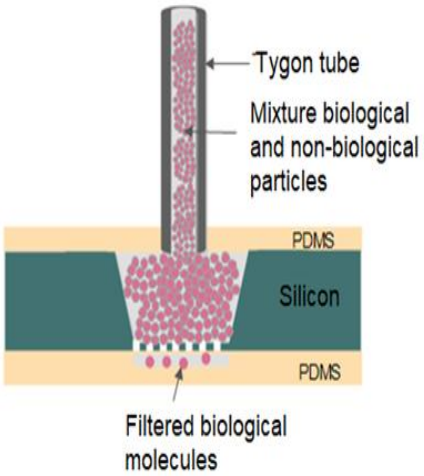

Fig. 2: Cross section of complete filtration system

3. Result and Discussion

The first stage of testing procedure, deflection of silicon membrane was tested using laser displacement meter to observe the capability of membrane due to the pressure applied. The simulation, calculation and measured deflection result of silicon membrane is compared as depicted in Fig. 3. From the graph show that the deflection was directly proportional to the applied pressure. The pressure 15 -55 mmHg used to mimic the filtration pressure in human kidney [11]. From the value of percentage error of simulation and experimental result show that the membrane thickness was suited to this application with the percentage error for simulation and experiment result is 0.86%. Furthermore, the membrane would not breakage during the testing procedure. So, membrane silicon is suited for this application and ready for testing procedure.

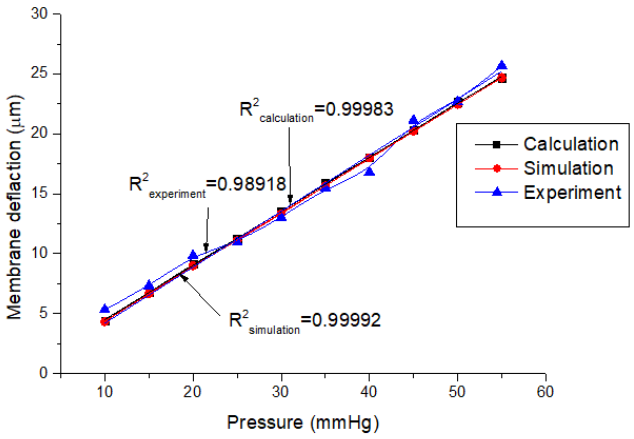

Fig. 3: Silicon membrane deflection for different pressure applied

After the capability of membrane has been studied, the pores were formed on silicon membrane surface. The characterization for pore formation by manipulating parameter likes current density, HF concentration, dopant type has been discussed in previous research [12]. The high current density was the finest parameter for columnar structure porous silicon using un-doped silicon material. The novelty of this paper was the low cost and simplest filtration technique to prove the functionality of nanoporous silicon to filter biological and non-biological particles. The study on columnar structure and small pore size has been studied using the dopant silicon [13-15]. But the pore structure was ease to control under impurity silicon compared to intrinsic silicon. Under aforementioned parameter the columnar structure and pore size 20-30 nm were formed to produce the filtration membrane system. To get 20-30 nm pore size under intrinsic silicon, the volume ratio of 5:5 between HF and ethanol as shown in Fig. 4 for different time. The pore size less than 100 nm can be formed under high concentration HF. In this case, 5:5 volume ratio has chosen to produce pore less than 100 nm by controlling the immersing time with high current density which is 600mA/cm^2. In this experiment setup, immersion time 5 minutes used to get pore size 20-30 nm and the pore penetrate the 5 μm silicon membrane.

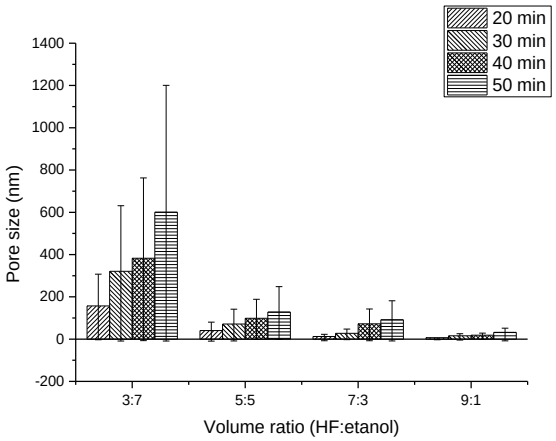

Fig. 4: The different HF concentration for different size of pore

In the first part of testing experiment setup, the size of particles likes indium oxide, protein, albumin and sodium chloride is measured in Zetasizer Nano SP for pre-filtration particles sizing and counts. The size of indium oxide was found to be between 100 – 500 nm. For biological particles used, the size of protein standard size has been measured between 4 to 11 nm, serum albumin 3-8 nm and sodium chloride clusters between 0.5 to 5 nm.

The particles that passed through the nanoporous silicon membrane were collected using the syringe and then inspected under the Zetasizer Nano to count the passed through the filter. 98.5 % of protein molecules was passed through the filtration membrane. The mean size of particles within samples was approximately 6.433 nm with a standard deviation across samples of 0.727 nm as depicted in Fig.5 (a).

The next sample of filter membrane used to filter out the albumin serum and indium oxide. From the graph in Fig. 5 (b) shows the particles of albumin serum with size less than 10 nm has been filtered out through this filter. The mean of particles has been passed through the membrane is 5.268 nm with standard deviation 0.7514 nm. By comparing the particle before and after the filtration shows that 100% has been passed through this filter membrane. According to the Fig. 5 (c) the sodium chloride particles had passed through this filter membrane with mean size particles 0.7128 nm with the standard deviation 0.1105 nm.

The results before and after filtration were inspected under EDX to identify the absence of indium oxide particles and sodium chloride molecules. The indium oxide and sodium chloride were observed before filtration system as depicted in Fig. 6. Meanwhile, Fig. 7 shows that sodium chloride molecules were passed through this filter membrane.

Fig. 5. The size of particles has been filtered using nanoporous silicon membrane for (a) Indium oxide and protein standard (b) Indium oxide and albumin serum (c) Indium oxide and sodium chloride

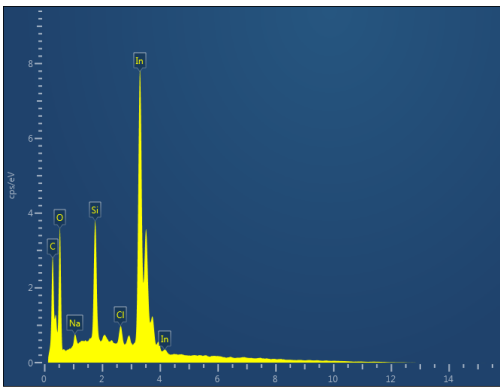

Fig. 6: Indium Oxide and Sodium Chloride before filtration

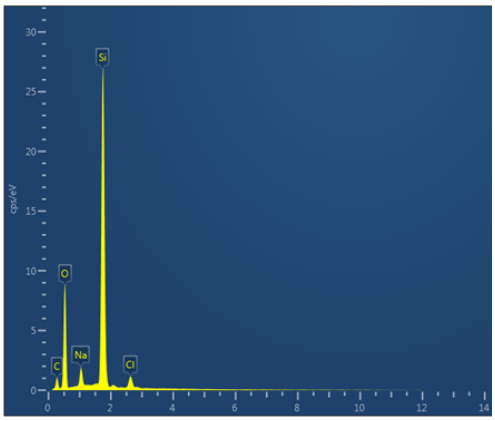

Fig. 7: After filtration membrane inspect the particle of Sodium Chloride only

This experiment setup observed nanoporous silicon membrane is capable for separating wanted and unwanted particles by observing and measuring the size of particles using Zetasizer Nano SP. This porous membrane can also be integrated and attached with other MEMS device to separate and filter the particles or biological molecules. The technique of filtration techniques is suitable to other material, but the pore size must follow the size of particles need to be filtered out.

4. Conclusion

Nanoporous silicon membrane have been successfully fabricated starts with thinning the silicon using KOH etching process followed by high current electrochemical etching process step. During the testing procedure, the indium oxide particle is mixed with the several biological solutions. It was observed that biological solution was separated with indium oxide using this filter membrane. More than 98% of biological molecules has been filtered out from this membrane by comparing the size of biological molecules before and after filtration. Due to this process, this filtration can be suggested to apply this simple and reliable fabrication process for artificial kidney application in the future.

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