SIZE-SPECIFIC SEPARATION OF BIO-MOLECULES USING POROUS ALUMINA MEMBRANE
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ABSTRACT
This paper reports an on-chip nano-porous membrane that can separate bio-molecules based on their sizes. The nano-porous membrane is one of key elements for the separation of bio-molecules. In conventional approaches, however, there are many limitations such as difficulty in integrating with a lab-on-a-chip (LOC) device and the lack of precision in separations. In this work, porous alumina membrane was directly fabricated on a microfluidic platform to be integrated with LOC. We demonstrate an effective, size-specific separation of DNA and protein with such device.

KEYWORDS: Bio-molecule Separation, Porous Alumina Membrane, Lab-on-a-chip

INTRODUCTION
An efficient bio-molecular separation through the nano-porous membrane requires the material with controllable pore size, length and surface chemistry. The pore size needs to be in the range of a few tens of nanometers. The size of pores should have a uniform distribution in order to achieve high selectivity of separation. Finally, high porosity is required for obtaining enough analyte flux. Commercially available nano-porous membranes generally exhibit large size distribution and relatively large thickness values [1]. Furthermore it is cumbersome to integrate such membrane with an on-chip device to construct a reliable one. Recently bio-molecule separation driven by diffusion or pressure using such approach have been demonstrated [2]. In order to take full advantage of on-chip microfluidic system it is necessary to develop fabrication process suitable to integrating the nano-porous membrane.

Here we describe an on-chip microfluidic device with built-in porous alumina membrane of precisely controlled nanopores. The nano-porous membrane was directly fabricated on a microfluidic platform resulting in an integrated architecture with a thin porous alumina membrane. The size of nano-pores has narrow size distribution. Pore widening and atomic layer deposition were used to precisely control the size. Bio-molecule separation through the nano-pores driven by electric potential was performed and characterized. We demonstrate that while single strand DNAs can easily pass through the porous alumina membrane, aptamer-protein complexes are effectively prevented from such separation due to their size.

EXPERIMENTAL
Porous alumina was adopted as a membrane material that enables bulk and low cost fabrication without high cost lithographic techniques such as nano-imprint lithography and e-beam lithography. Highly dense and uniform pores of porous alumina spontaneously formed from aluminum layer by an anodizing process. Porous alumina membrane was directly fabricated on silicon substrate using micro fabrication process (Fig. 1(a)). Nitride membrane was suspended by KOH anisotropic wet-etching, then titanium and aluminum were deposited by a metal evaporator with the thickness of 50 nm and 1 μm, respectively. The first anodizing process was carried out in 0.3 M oxalic acid (H₂C₂O₄) at 40 V. Pores were formed in a relatively regular shape as the anodizing time elapses due to the self-alignment of stressed pores [3]. 6 wt% phosphoric acid (H₃PO₄) and 8 wt% chromic acid (H₂CrO₄) were mixed at 60 °C and used to selectively remove the first alumina layer. Periodic vertical pores were formed during second anodizing process with same condition as the first step. The uniformity of the pore size is critical to the accurate separation of molecules based on their sizes. Pores were slightly widened to immerse the porous alumina membrane into 10 wt% phosphoric acid at 30 °C for 15 min to enhance uniformity of pores. Etching rate of widening process was about 0.8 nm/min at room temperature. For a controllable size of pores, Ruthenium(Ru) was deposited by an atomic layer deposition (ALD) process. Finally, several steps of top/back side RIE etching were performed to open back side of the membrane.

Fig. 1(b) shows the configuration of a single chip device that includes two PDMS chambers between porous alumina membrane-silicon substrate. Each chambers consist of two reservoirs, venting holes and microfluidic channels. The upper chamber is directly connected to the bottom chamber so that a sample injected is spontaneously flows into the bottom chamber via the hole in the silicon substrate by capillary effect. The PDMS structures were effectively bonded to porous alumina membrane and silicon substrate through O₂ plasma treatment.
Scanning electron microscope (SEM) images were taken to inspect the porous alumina membrane. Fig. 2 (a) shows the porous alumina membrane that suspended on the selectively etched silicon substrate. Fig. 2 (b) shows the magnified image of the membrane. Average diameter of vertically formed alumina pore was about 38 nm and thickness of the membrane was 500 nm. Pores penetrate up to back side of the alumina membrane, and well opened pores were shown in Fig. 2 (c). Pore size distribution was confirmed by image analysis with Vision Assistant (National Instrument, USA). Standard deviation (6.64 nm) of pore size is better than that of the pores fabricated by rapid thermal annealing (RTA) process[2]. In case of Ru coated pores, average diameter of pores are reduced to 13 nm with 450 cycles of ALD process.

RESULTS AND DISCUSSION

Pt wires were fixed in each reservoir and connected to the power supply to set electrophoretic experiment. 50 μl of TAE buffer was filled inside the upper chamber, and then 50 μl of TAE buffer with fluorescine isothiocyanate (FITC) tagged single strand DNAs were injected into the bottom chamber. Due to capillary effect and venting holes, the injection was possible without any external pressure source. Electric potential was applied across the porous alumina membrane and ssDNAs in the bottom chamber moved into upper chamber via nano-pores on the membrane driven by electrophoretic forces. Separated DNAs, located below the membrane, appeared on the upper chamber and were observed by fluorescence light intensity on a microstage. Complex of thrombin (coagulation protein, 36 kDa) and thrombin aptamer (4 kDa) were prepared to perform a size specific bio-molecule separation experiment. 1 μM of thrombin and FITC (0.33 kDa) tagged thrombin aptamer were incubated in the aptamer binding buffer (20 mM Tris-acetate pH : 7.4, 140 mM NaCl, 5 mM KCl, 1 mM CaCl2, 1 mM MgCl2) for 1 hour to make aptamer-thrombin complex. Separation experiments were performed in various voltages and buffer conditions.

Fluorescence intensity of aptamer-thrombin complex and control DNA(15 mer poly-A) were compared. Fig. 3(Left) shows that aptamer-thrombin complexes were rarely separated while poly-A effectively emerged to upper chamber because of its molecular size. Although light intensity of aptamer-thrombin complex is lower, it seems that a small amount of complex molecules were separated and we guess they are non-bind aptamers (free aptamers). Light intensity was quantified by image analysis using computational method and maximum light intensity of each molecules in various voltages were plotted in Fig. 3(Middle). Separation efficiency of poly-A is much higher than aptamer-thrombin complex at 4.5 V. Finally, the mixture of aptamer-thrombin complex and rhodamine (red fluorescence) tagged poly-A were introduced. Light intensity of red fluorescence and green were initially normalized to one for equivalent comparison and shown in Fig 3(Right). It was found that even green light (aptamer-thrombin complex) intensity decreased while red light intensity increased. It is because complex molecules, located below the membrane, were covered by separated poly-As.

Figure 1. (a) Fabrication process of a freestanding AAO nano porous membrane,(b) Configuration of complete lab-on-a-chip device.

Figure 2. SEM images of AAO nano-porous membrane and complete device. (a) Free standing AAO membrane on silicon substrate. (b) Magnified view of AAO membrane. (c) Barrier layer of AAO was etched to open pores. (d) Ru coated pores.
CONCLUSION
We fabricated a nano-pore LOC device that can effectively separate bio-molecules by sizes. A variety of applications will be enabled where a size-dependent separation of molecules is needed. Without the needs of high temperature and exotic fabrication steps, the device can be easily modified and adapted to various LOC platforms which require only a small amount of sample.

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REFERENCES

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