Permeability and Protein Separations: Functional Studies of Porous Nanocrystalline Silicon Membranes

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ABSTRACT

We have developed an ultrathin (15nm) freestanding nanoporous silicon based membrane. This new material, termed porous nanocrystalline silicon (pnc-Si), is fabricated using standard photolithography techniques. The intrinsic characteristics of this new material and its relatively facile fabrication make it superior to commercial membranes and other currently studied nanomembranes. We have assembled these membranes into simple diffusion and centrifugal apparatuses. We show that we can achieve sharp protein separations of concentrated complex protein mixtures. We additionally show that air and water permeabilities through pnc-Si membranes are greater than both polycarbonate track etched membranes and carbon nanotube membranes.

Keywords: silicon, membrane, ultrathin, separation, protein

1 INTRODUCTION

Clinical, laboratory, and industrial research frequently make use of nanoporous membranes, and the demand for such a membrane continues to grow. These membranes are used to perform molecular separations by size and charge [1]. An ultrathin membrane with well-defined monodisperse pores would be the ideal instrument for molecular separations. Unfortunately, there are no commercially available membranes that are nanoporous and have a nanoscale thickness.

Polymeric membranes are the most common nanoporous membranes used for molecular separations, and these are made from materials such as cellulose and polyether sulfone [2]. These membranes are employed to perform procedures such as hemodialysis and are a common material in laboratory spin columns. They are referred to as tortuous path membranes since they have a mesh-like morphology and lack discrete pores. Polymeric membranes have thicknesses on the order of hundreds of micrometers to millimeters, and the large surface area of the winding pathways causes sample loss and biofouling due to protein adsorption [2]. The molecular weight cutoff for these membranes is approximate due to the lack of well-defined cylindrical pores. Polymeric membranes function best by separating molecules that differ in weight by whole magnitudes. These membranes, though, are easily and inexpensively made, and perform well as an initial rough purification step.

While nanomembranes can be engineered to have controlled pore sizes and are able to perform sharper separations, those that are commercially available are still thick and have long cylindrical pores. Track etched membranes (Whatman, Sterlitech) are manufactured by bombarding a thin plane (~6 μm) of polycarbonate or polyester with high energy ions and subsequently etching pores where bonds were severed. This creates membranes with pore sizes between .01 and 20 μm [2]. The pores are generally well defined, but porosity must be kept low to reduce the chances of pores overlapping and creating larger holes in the membrane. Pores self assemble by anodization in alumina membranes (Anopore, Whatman), and by changing the voltage of anodization, the pore size of these membranes can be tuned between 20 and 200 nm [3]. The

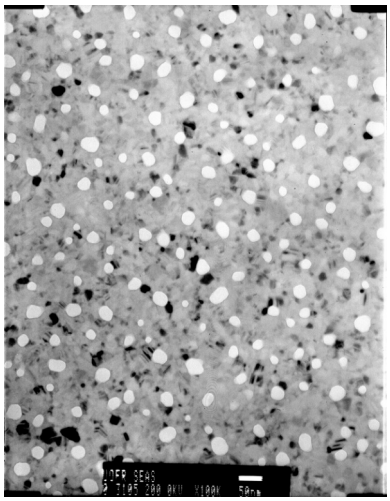

Figure 1 - TEM image of 15 nm thick porous nanocrystalline silicon membrane. The film is composed of silicon nanocrystals oriented in different directions; those that satisfy the Bragg conditions appear as black spots upon the gray film. Pores in the membrane appear as white circles. Pore diameter and porosity are measured directly from TEM micrographs.

porosities are higher than track etched membranes, and the pores are very homogeneous. Both track etched and alumina membranes are convenient to manufacture, but their relative thickness means increased resistance to fluid flow [4] and a larger surface area for protein adsorption. Porous silicon nanomembranes and carbon nanotube membranes [5] are currently being researched, but both of these membranes also have cylindrical pores and face the same problems as commercially available engineered nanomembranes.

We have developed a novel ultrathin nanomembrane with well-defined disc shaped, rather than cylindrical, pores [6]. The material, called porous nanocrystalline silicon, is fabricated using standard photolithography techniques. Pore sizes can be tuned in a thermodynamically driven self-assembly step during pnc-Si manufacture. Because of the thinness of the material, we can readily measure porosities and the diameters of the well-defined pores by TEM (Figure 1). The intrinsic characteristics of this new material and its relatively facile fabrication make it superior to commercial membranes and other currently studied nanomembranes. We have built apparatuses to test the membrane in both diffusion and pressurized flow modalities. We have studied the permeability and performed separations of concentrated complex protein mixtures using this novel nanomembrane.

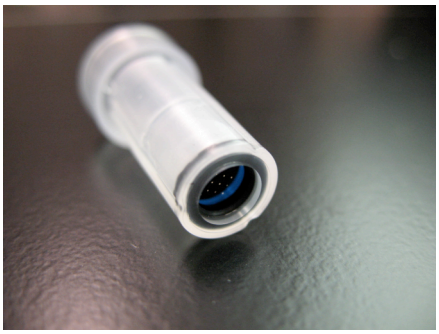

Figure 2 – Centrifuge tube insert. Circular silicon chips with 2 pnc-Si silts are sandwiched between and o-ring and a polypropoline retention ring, sealing it inside the polypropoline insert. These inserts are placed in a 1.5 mL centrifuge tube and filled with the solution to be tested and spun in a centrifuge.

2 METHODS

2.1 Membrane Fabrication

Membranes were fabricated as discussed in [6]. We used a photolithography mask that allowed us to create a wafer with 64 .6 cm chips, each containing two .5 mm by 2 mm slits spanned with pnc-Si membrane material.

2.2 Diffusion Apparatus and Protein Separations

We built four ~55 uL reservoirs in a block of erylite. We filled each reservoir with 60 uL of phosphate buffered saline (PBS) so that the fluid curved slightly above the reservoir. We placed a membrane chip above each reservoir so that the membrane came in contact with the PBS. A 2 ul drop of 10mg/mL bovine brain extract was pipetted into the well of the silicon chip. Diffusion of the protein solution was allowed to occur for 24 hours.

After 24 hours we recovered the retentate, or the solution remaining above the membrane, and the filtrate, or the solution in the reservoir. Both retentate and filtrate were analyzed using SDS polyacrilimide gel electrophoresis (SDS-PAGE) and were compared to a control solution that did not see the membrane.

2.3 Centrifuge Tube Apparatus

Harbec Plastics (Ontario, NY) built a polypropoline centrifuge tube insert that would enable us to perform centrifugation using our pnc-Si membrane (Figure 2). The membrane is sealed in the tube with an o-ring and plastic retention ring. Solutions to be tested can be pipetted into the insert and will pass through the membrane during centrifugation.

2.4 Permeability Testing

Water permeability was tested using the centrifuge tube insert. 500 uL of water was pipetted into the device and the underside of the membrane was wetted using 200 uL water. The device was spun in a 15 mL conical at 1000 rpm for 2 hours. The volume of water to pass through the membrane was measured and the permeability (volume per time per active membrane per psi) was calculated.

Air testing was performed using the setup as illustrated in Figure 3. The volume of nitrogen to pass through the membrane was measured using the scale on a horizontal u-tube, and the permeability was calculated.

3 RESULTS AND CONCLUSIONS

In Figure 4 we show that we obtained a sharp separation of the 10 mg/mL bovine brain extract. Proteins under 90 kD begin to appear in the filtrate, and the lowest molecular weight species are hardly detected in the retentate. 30kD proteins are sharply separated from 90kD proteins in this gel, and this represents only a 3x difference in size. Protein size effects and absorption will effect the cutoffs that we see for different protein mixtures, and this is an area of ongoing research.

We plot the permeability values we obtained against two engineered nanomembranes, polycarbonate track etched membranes and carbon nanotube

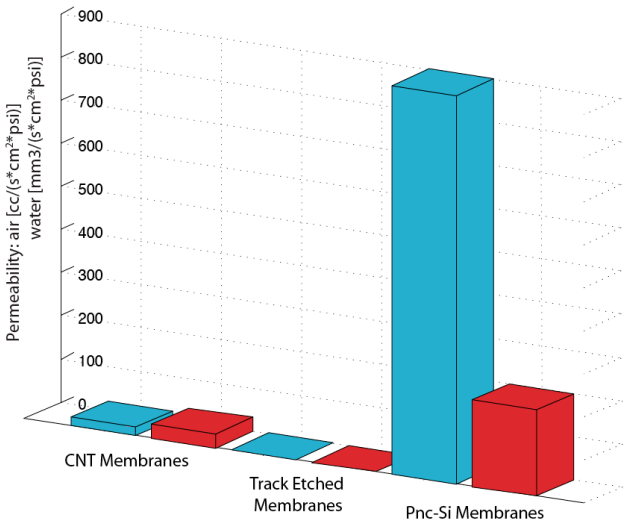

Figure 3 – Air and water permeability of nanomembranes. Here we contrast the permeabilities of CNT membranes and track etched (TE) membranes from [5] with the permeabilities of pnc-Si membranes. Pnc-Si membranes are an order of magnitude thinner than CNT and TE membranes, and we show here that there is less resistance to air and water flow through pnc-Si membranes.

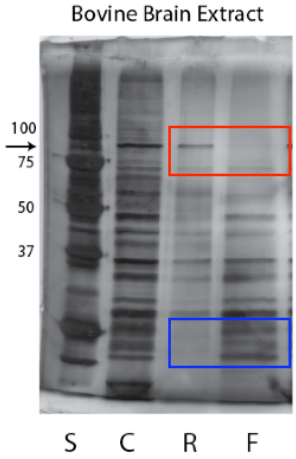

Figure 4 – Brain Extract Separation. S = Molecular Weight Standards, C = Control (protein that has not seen the membrane), R = Retenetate, F = Filtrate. Here we show a fractionation of 10 mg/mL bovine brain extract. The red box outlines a ~90kD protein that appears in the retenetate, but not the filtrate. The blue box outlines a series of low molecular weight proteins that appear more heavily in the filtrate. We consider our cutoff to be around 75k in this separation.

membranes [5]. Permeability of air and water is a magnitude higher though pnc-Si membranes, as expected due to the relative thickness of track etched and nanotube membranes.

In conclusion, we show that the characteristics of pnc-Si membranes enable them to perform better than other current nanomembranes. Sharp molecular separations are achieved because of the well-defined pores and lower surface area for adsorption. The air and water permeaility of pnc-Si membranes is much greater than cylindrical pore membranes, and presumably so will be the molecular transport. In the future we plan to study the effects of pore distribution on molecular weight cutoffs and the efficiency of filtration through pnc-Si membranes.

REFERENCES

[1] M. Menon and A. Zydney. J. Mem. Sci, 181, 179-184, 2001.

[2] C. van Rijn. "Nano and micro engineered membrane technology." Volume 10. Elsevier, 2004.

[3] R. Furneaux, W. Rigby, and A. Davidson. Nature, 337, 147-149, 1989.

[4] C. van Rijn, M. van der Wekken, W. Nijdam, and M. Elwenspoek. J. Microelectromechanical Sys, 6, 48-54, 1997.

[5] J. Holt et al., Science, 312, 1034-1037

[6] C. Striemer, T. Gaborski, J. McGrath, and P. Fauchet. Nature, 445, 749-753, 2007.