Nanoporous Membrane with Ultrahigh Selectivity and Flux Suitable for Filtration of Viruses

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Introduction

Filtration, separation and isolation of viruses have been critical issues in controlling blood-borne viral infection and in virus researches[1-2]. Membrane-based technology has been recognized as a useful method for separation of bio-materials including viruses due to its efficiency, ease of implementation and cost-effectiveness. Several types of membranes have been employed for filtration of virus with tens of nanometers in size. However, Ultra-filtration (UF) membranes have not been effective, since virus particles permeate into a small number of abnormally large-sized pores. Track-etched polycarbonate and anodized aluminum oxide (AAO) membranes with uniform pore size showed very low flux for the virus separation. Here, we introduce a new membrane with an asymmetric film geometry that shows both high selectivity and high flux. This membrane consists of thin nanoporous layer, prepared from a block copolymer template, and a support membrane that provides mechanical strength. This asymmetric membrane shows ultrahigh selectivity while maintaining high flux for the separation of human rhinovirus type 14 (HRV14), having a diameter of ~ 30 nm, a major pathogen of the common cold in humans.

Experimental

Thin films (~80 nm) of the mixture of PS-*b*-PMMA with molecular weight of 89,000 g/mol and 10 wt.-% homopolymer PMMA (Mw = 30,000 g/mol) relative to the PMMA block were prepared by spin coating of 2% (w/v) toluene solutions onto the modified silicon wafers and then annealed at 170 ℃ under vacuum for two days, and quenched to room temperature. The films were floated onto the surface of a 5 wt% HF solution, transferred to a water bath, then transferred to porous membrane supports (HT Tuffryn™, Pall Life Science) having an average diameter of 0.2μm and a thickness of 150μm. The PMMA homopolymer was removed by washing with acetic acid for 60 min at room temperature. The nanopores in the thin film were observed by field emission scanning electron microscopy (FE-SEM, Hitachi S-4200). Permeation experiments were performed at a stirring speed of 200 rpm and a pressure of 0.1 bar at room temperature in a stirred cell module (Amicon 8010, Millipore Co.). The morphology of deposited virions on the membranes was investigated by scanning force microscopy (SFM, Digital Instrument D3000) with silicon nitride tips on cantilevers (Nanoprobe) in the tapping mode and by FE-SEM. Cultivation and purification of HRV14 was performed as described by Erickson et al.[3] A virus solution (5 ml) [5x10^6 PFU/ml in phosphate buffered saline (PBS)] was forced to pass through various filters, and then plaque assays[4] were performed using solutions penetrated through the filters.

Results and discussion

Figure 1 shows a schematic diagram of the fabrication of asymmetric nanoporous membranes. The top separation layer (~ 80 nm thick) is made from a thin film of a mixture of polystyrene-*block*-poly(methyl methacrylate) copolymer (PS-*b*-PMMA), having cylindrical microdomains of PMMA, on a ~ 100 nm thick sacrificial silicon oxide layer. As previously reported,[5] when PMMA homopolymer is added to PS-b-PMMA, the cylindrical nanodomains orient normal to the surface in films up to ~ 300 nm in thickness on surfaces where interfacial interactions have been balanced[6] (Figure 1a). This thin film was removed from the substrate by using a buffered HF solution to dissolve the oxide layer. It was then transferred onto the MF polysulfone (PSU) membrane that acted as a support (Figure 1b). The adhesion between the block copolymer mixture film and PSU membrane was enough to maintain the mechanical strength during the fabrication and the filtration experiment. Porous thin films of the upper layer were prepared by selectively removing the PMMA homopolymer from the cylindrical PMMA microdomains with acetic acid (Figure 1c). This produced a well-ordered array of ~15 nm diameter pores having a narrow pore size distribution which completely block HRV 14 virus (colored in green) to penetrate into pores, while proteins, such as bovine serum albumin (BSA) (colored in yellow) with a size of ~7.2 nm, freely passed through the pores in the membrane (Figure 1d).

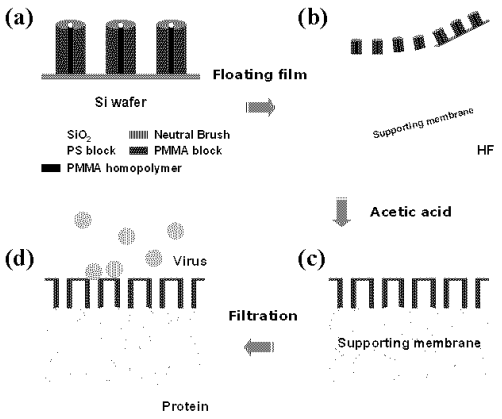

Figure 1. The procedure for the fabrication of asymmetric nanoporous membrane.

Conclusions

We describe a new double layered nanoporous membrane suitable for virus filtration. One layer is an 80 nm thick film having cylindrical pores with diameters of 15 nm and a narrow pore size distribution. This layer is prepared by using a thin film of the mixture of a block copolymer and a homopolymer, and mainly acts to separate viruses. The support layer (~ 150 microns thick) is a conventional micro-filtration membrane with a broad pore size distribution. This asymmetric membrane showed very high selectivity and flux for the separation of human rhinovirus type 14 (HRV 14) which has a diameter of ~ 30 nm and is a major pathogen of the common cold in humans. This virus filter can be applied to the cultivation of human cells for therapeutic purposes using animal sera without risk of infection of zoonotic viruses. Moreover, it is possible to develop a virus-proof filter for haemodialysis of patients with renal failure who are at great risk of viral infection.

References

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