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Removal of airborne bacteria by filtration using a composite microporous membrane made of a pyridinium-type polymer showing strong affinity with microbial cells

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SUMMARY

A composite microporous membrane made of poly(*N*-benzyl-4-vinylpyridinium chloride) that showed strong affinity with bacterial cells was prepared as a filter material for removing airborne bacteria. Thickness, pore diameter and porosity of the membrane were 0·72 mm, 14·5 μm and 63 %, respectively. Electron micrographic analysis revealed that the membrane consisted of a very large number of connected beads of 1·4 μm in diameter made of the pyridinium-type polymer. Filtration using the membrane was performed easily at low flow rates with insignificant pressure drop across the membrane. Filtration at 63·7 cm/sec gave 99·98 % and 99·996 % removal (3·7 and 4·4 $\log_{10}$-unit reduction in concentration) of *Escherichia coli* and *Pseudomonas aeruginosa*, respectively. *Staphylococcus aureus* was not detected in filtrates. Since pores of the membrane were much larger than these bacteria, the efficient removal was best explained in terms of the affinity of the polymer with bacterial cells.

INTRODUCTION

Protection of interior air from microbial pollution is important for public health and hygiene. The control of nosocomial infection due, for example, the methicillin-resistant *Staphylococcus aureus* (MRSA) is of increasing concern and has led to a renewed interest in the control of contamination within the hospital environment. In certain circumstances the removal of airborne micro-organisms is required and conventionally this is achieved by filtration. Filter materials that prevent physical penetration of particles larger than 0·2 μm are generally used for this purpose, because sizes of the organisms to be removed are in the range 0·1–10 μm. However, filtration using such materials requires appreciable pressure. For practical uses, filtration with an insignificant pressure drop across the filter would be desirable, allied to high removal efficiency.

In a previous publication from our laboratory [1], we reported on the excellent performance of a pyridinium-type polymer for the removal of micro-organisms from water. This functional polymer was proposed as a filter to protect drinking water supplies from microbial pollution. Non-woven cloth coated with a small amount of the pyridinium-type polymer was found to be efficient for removing bacteria and spores of fungi from water [2]. Conventional filtration through ten sheets of the coated cloth at 300 cm/h removed 99 % of bacteria. The coated cloth allowed pressure-free filtration owing to its large pores (about 540 μm in diameter). However, the filtration showed high removal efficiency, probably due to a special affinity of polymer for microbial cells [1, 3, 4].

In this work we have attempted to remove airborne bacteria by filtration with insignificant pressure drop across a filter material made of the same polymer.

MATERIALS AND METHODS

Materials

4-Vinylpyridine was purified by distillation under reduced pressure. Styrene and 55 % divinylbenzene

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were purified by washing with 5% aqueous sodium hydroxide solution, followed by distillation under reduced pressure. 2,2′-Azobisisobutyronitrile (AIBN) and other chemicals and solvents were used without further purification. Non-woven cloth, 0·5 mm thick, made of pure 1·5 denier rayon was provided by Japan Vilene Co. Ltd. (Tokyo, Japan).

Non-woven cloth coated with a pyridinium-type polymer

Polymerization was carried out in a 500 ml, round-bottomed, three-necked flask equipped with a mechanical stirrer, a reflux condenser, and a gas inlet. A mixture of 4-vinylpyridine (28·1 g, 0·267 mol), styrene (76·3 g, 0·733 mol), and AIBN (0·73 g, 4·5 mmol) was added to 250 ml of ethanol under a nitrogen atmosphere and heated at 80 °C with stirring for 6 h. After cooling to room temperature, benzyl chloride (33·8 g, 0·267 mol) was added, and the mixture was allowed to react at 80 °C for 5 h. After the reaction, poly(*N*-benzyl-4-vinylpyridinium chloride-*co*-styrene) was isolated by pouring the contents of the flask into ethyl acetate, and was dried *in vacuo* to constant weight. Intrinsic viscosity of the polymer was 0·25 dl/g when determined in ethanol containing 10 g/l of $MgCl_2.6H_2O$ at 30 °C. Elemental analyses showed that the polymer contained a 2:5 molar ratio of *N*-benzyl-4-vinylpyridinium chloride and styrene, and 1·77 mmol/g of *N*-benzyl-4-vinylpyridinium chloride.

Non-woven cloth was coated with 40 mg/g of the pyridinium-type polymer as reported previously [2].

Composite microporous membrane made of cross-linked poly(*N*-benzyl-4-vinylpyridinium chloride) and reinforced by non-woven cloth

A 13-cm square sample of the non-woven cloth was soaked in a monomer mixture containing 4-vinylpyridine (30 g, 0·285 mol), 55% divinylbenzene (3·0 g, 0·013 mol), AIBN (326 mg, 2·0 mmol), toluene (37·5 g), and acetone (7·5 g) for 10 min at room temperature. The treated cloth was placed on a Teflon sheet supported by a mirror glass of 7 mm thickness, and several drops of the monomer mixture were added. After degassing, the treated cloth was covered by another Teflon sheet supported by a mirror glass. Sandwiched cloth was fastened using clippers and placed in a water bath. The water bath was placed in a 10-litre pressure cooker. Polymerization was performed by heating and pressure cooker to about 120 °C for 30 min. After washing with deionized water and drying at room temperature, the membrane was placed in a 2-litre Erlenmeyer flask containing benzyl chloride (50 g, 0·39 mol) and ethanol (1·5 litre) at pH 7. The mixture was allowed to react at 70 °C for 6 h to produce a composite microporous membrane made of cross-linked poly(*N*-benzyl-4-vinylpyridinium chloride) and reinforced by non-woven cloth. After drying to constant weight at room temperature, the membrane was extensively washed by passing deionized water through the membrane until total organic carbon from the filtrate disappeared. The membrane contained 0·36 mol/m^2 of the pyridinium group determined as reported previously [5]. Mean pore diameter and porosity of membranes were determined by electron microscopy.

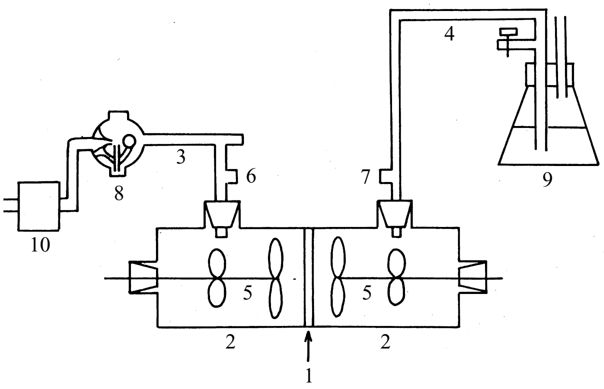

Fig. 1. Apparatus used for the experiments of removal of airborne bacteria. 1, the filter material; 2, glass column; 3, glass inlet; 4, glass outlet; 5, mechanical stirrer; 6, tuber for sampling of the influent; 7, tube for sampling of the effluent; 8, nebulizer; 9, Erlenmeyer flask containing disinfectant solution; 10, air pump.

Bacteria

Escherichia coli IFO 13168, *Pseudomonas aeruginosa* IFO 3080 and *Staphylococcus aureus* IFO 3060 were incubated for 20 h at 37 °C. The nutrient broth used for the culture of these bacteria was prepared by dissolving 3·0 g of meat extract and 5·0 g of peptone into 1000 ml of water at pH 7·0. Bacterial cells were harvested by centrifugation at 2000*g* for 20 min at room temperature, and washed repeatedly with sterile physiological saline followed by centrifugation at 2000*g*.

Removal of airborne bacteria by membrane filtration

All procedures were performed under aseptic conditions. Filtration experiments were performed with a glass apparatus as shown in Figure 1. The filter material (1) was placed between two glass columns (2) connected with a stopper made of silicon rubber and a glass inlet (3) or outlet (4), and a mechanical stirrer made by remodelling a hypodermic syringe (5). The mechanical stirrer was used as a fan to achieve a uniform distribution of bacteria in air. Its speed of rotation was 120 rev/min, and the inner diameter of the tubing was 5 mm. The mechanical stirrer was used to prevent losses of bacteria by impingement on the surfaces, although complete exclusion of losses was difficult under the experimental conditions. The glass column (2) was 1·0, 1·5, 2·0, 3·0 or 5·0-cm inner diameter and 17-cm long. Volume of passed air per unit time was fixed throughout each experiment by fixing the operating conditions of the air pump. The rate of filtration (cm/s) was varied by changing the diameter of the filter that depended upon the inner diameter of the glass column. The glass inlet (3) and outlet (4) was connected with tubes for sampling (6) and (7). The glass inlet (3) was connected to a nebulizer (8) and an air pump (10). A conventional air pump generally used for water tanks or basins for tropical fish or goldfish was used in this work. The glass outlet (4) was connected to an Erlenmeyer flask (9) containing a disinfectant solution.

Aqueous suspensions of bacteria were placed in the nebulizer (8). Nebulization of the suspension formed a mist containing bacteria which was used as an airborne suspension of the bacteria. After filtration experiments, samples of the airborne suspension were removed using a hypodermic syringe, and immediately submitted to measurement of the concentration of viable bacteria. The percentage removal of viable bacteria was based on the difference between viable cell concentrations before and after filtration. Five samples of inflow and effluent air were taken in each experiment. Numerical values of the bacterial concentrations fluctuated as expected for a spread-plate method, but the order of concentration was usually not changed.

Measurement of concentration of airborne bacteria

After the filtration experiments, 10 or 100 ml portions of air suspensions of bacteria were withdrawn from the tubes for sampling (6 and 7) using a hypodermic syringe equipped with a needle, and immediately blown slowly into 1·0 ml of sterilized physiological saline. The aqueous suspensions obtained were subjected to measurement of viable bacterial cell concentration by decimal serial dilutions in sterilized physiological saline. From the dilutions, surviving bacteria were counted on nutrient media by the spread-plate method using incubation at 37 °C for 24 h. Growth medium was prepared by dissolving 10 g peptone, 10 g sodium chloride, 3 g yeast extract and 15 g agar in 1000 ml sterilized distilled water, and pH was adjusted to 7·0 before sterilization by autoclaving at 121 °C for 20 min. Figure 2 shows the relationship between the concentration of airborne *E. coli* and the turbidity of the suspensions placed in the nebulizer (8). The proportional relationship shown in Figure 2 supports the reliability of the testing method.

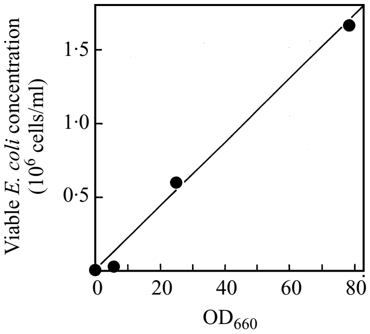

Fig. 2. Relation of viable cell concentration of airborne *E. coli* to turbidity at 660 nm of the suspension placed in the nebulizer.

RESULTS

The composite microporous membranes

During the polymerization procedure, an appropriate amount of solvent was used to make the resultant membrane microporous. After polymerization, the solvent was removed from the membrane by drying, and micropores appeared in the membrane. Weight ratio of monomers to solvents was an important factor determining porosity and pore size of the membrane. Characteristics of composite microporous membranes constructed with various ratios of monomers to solvents are listed in Table 1. Where the weight ratio of monomers to solvents was 8:2 and 7:3, the resultant membranes (designated membranes A and B respectively) strongly resisted the flow of nebulized air, and were not suitable as filter material for this work. Electron micrographs of these membranes did not show the presence of pores. Amounts of solvent were insufficient in these ratios.

Where the weight ratio of monomers to solvents was 6:4 and 5:5 (designated membranes C and D respectively), membranes permitted the flow of nebulized air. However, filtrations using these membranes

Table 1. *Properties of composite microporous membranes made of cross-linked poly*(N-*benzyl-4-vinylpyridinium chloride*) *and reinforced by non-woven cloth*

Membrane	A	B	C	D	E	F	G*
Thickness (mm)	1·43	1·07	0·95	0·83	0·72	0·49	0·53
Porosity (%)	—†	—†	36	42	63	74	51
Mean pore diameter (μm)	—†	—†	11·8	14·3	14·5	16·5	13·7
Mean particle diameter of beads (μm)	—†	—†	1·9	1·7	1·4	1·4	1·2

* Composite microporous membrane made of cross-linked poly(4-vinylpyridine) and reinforced by non-woven cloth.

† Pores were not observed.

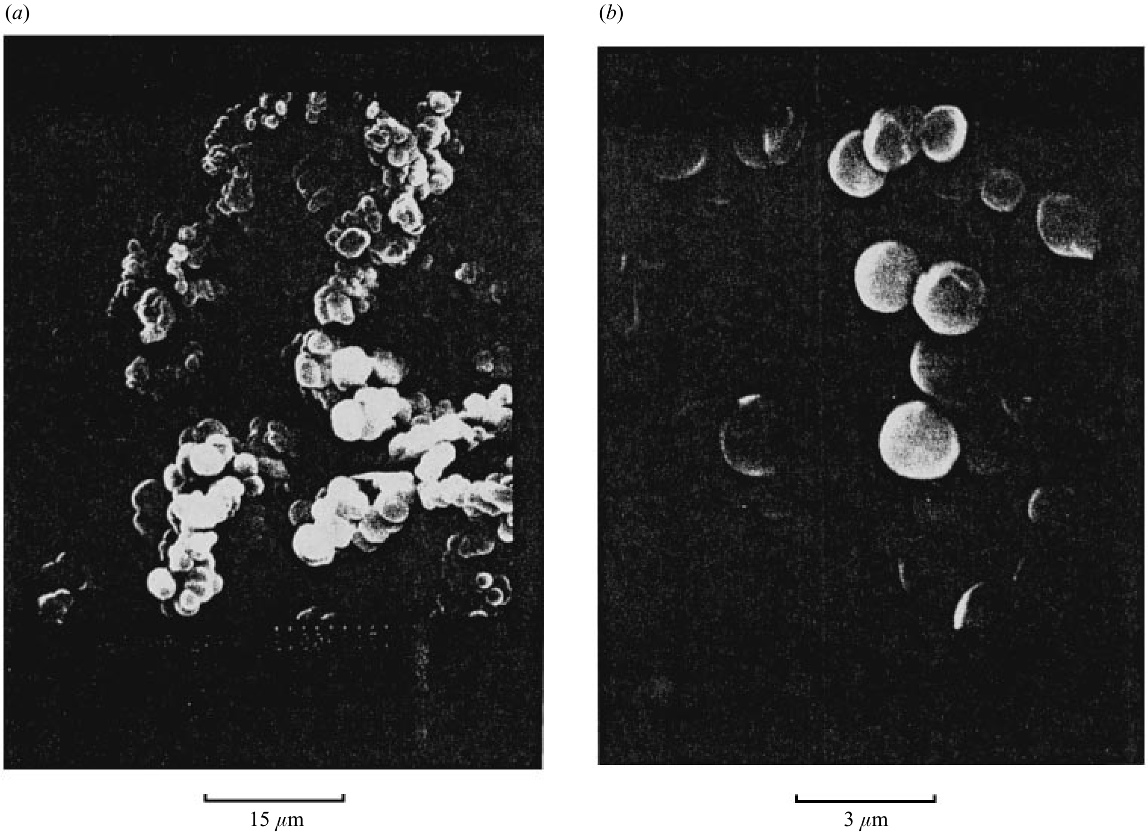

Fig. 3. Electron micrographs of membrane E.

were difficult. A porosity range of 36–42% seemed to be too low to achieve air filtration with insignificant pressure drop across the filter.

Where the weight ratio of monomers to solvents was 4:6 and 3:7, the resultant membranes (designated membranes E and F respectively) made easy filtration possible. Filtration using membrane E was highly efficient at removing airborne bacteria, but that using membrane F was not very efficient. Therefore, we used mainly membrane E in this work.

Electron micrographs of membrane E are shown in Figure 3 and pore diameter and porosity in Table 1. Membrane E was found to consist of a very large number of connected beads of cross-linked poly(*N*-benzyl-4-vinylpyridinium chloride) with a mean particle diameter of 1·4 μm (Table 1).

Where the weight ratio of monomers to solvents was 2:8 and 1:9, a membrane was not obtained. Electron micrographs of the products showed the presence of small numbers of beads of the polymer adhering to the surface of fibres of the non-woven cloth.

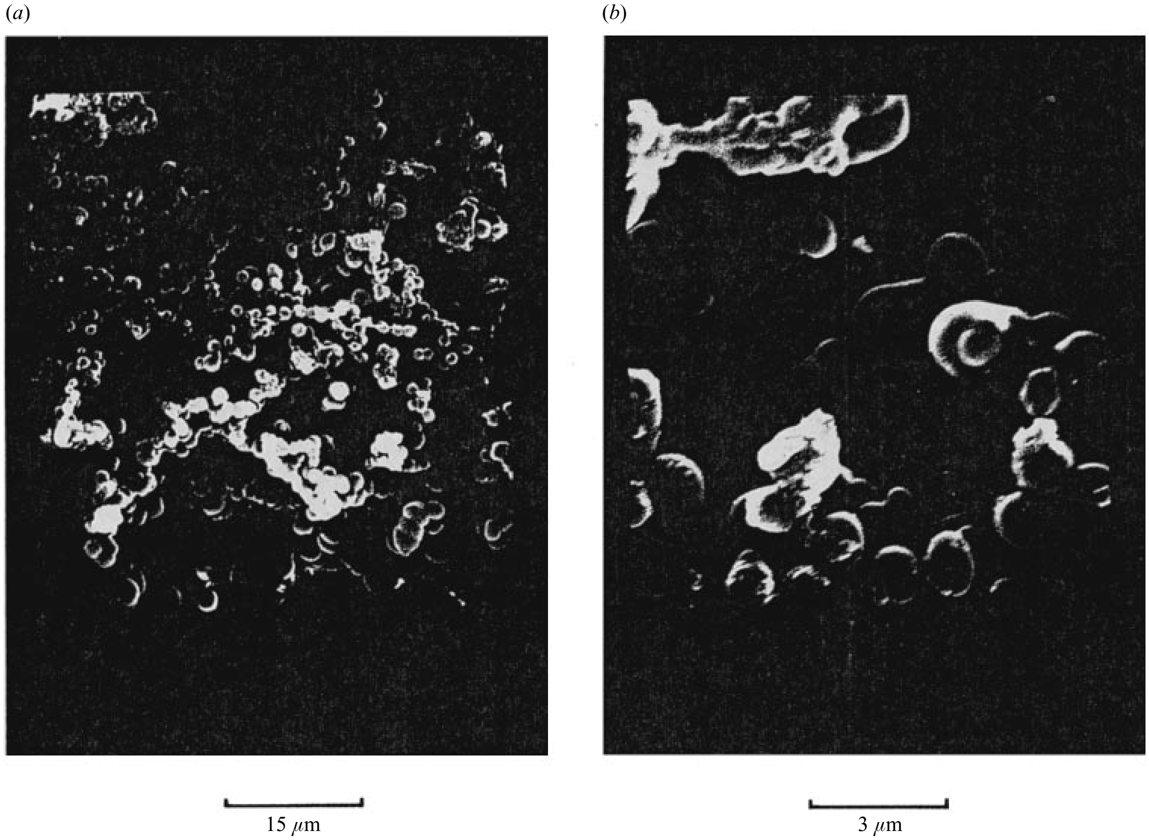

Fig. 4. Electron micrographs of membrane G.

For comparison, a composite microporous membrane made of cross-linked poly(4-vinylpyridine) and reinforced by non-woven cloth (designated membrane G) was prepared. Electron micrographs of membrane G are shown in Figure 4 and membrane characteristics in Table 1.

Removal of airborne bacteria by filtration using a composite microporous membrane made of cross-linked poly(*N*-benzyl-4-vinylpyridinium chloride)

Removal of airborne bacteria by filtration was performed using membrane E as an example of the composite microporous membrane and *E. coli* as a test bacterium. Nebulized suspensions of the bacteria were passed through the membrane using the filtration apparatus shown in Figure 1.

Figure 5 (1) shows the percentage removal expressed as a function of filtration rate. During slow filtration at 2·6 cm/s, 99·9997 % of the bacteria were removed (5·5 $\log_{10}$-unit reduction in concentration) from the air suspensions. During rapid filtration, the percentage removal was reduced, but remained at a level of 99·98 % (3·7 $\log_{10}$-unit reduction in concentration) even at a filtration rate of 63·7 cm/s.

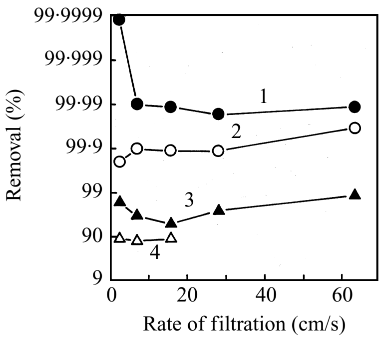

Fig. 5. Influence of the rate of filtration on the percentage removal of airborne *E. coli*. Inflow concentration of airborne *E. coli* was 1·16–3·33 × 10^5 cells/ml.

When membrane F was used at a filtration rate of 63·7 cm/s and at an inflow concentration of 1·10 × 10^5 cells/ml, 99·8 % of the bacteria were removed (2·7 $\log_{10}$-unit reduction in concentration).

Removal of airborne bacteria by filtration using a composite microporous membrane made of cross-linked poly(4-vinylpyridine)

Removal of airborne bacteria by filtration was performed in a similar manner using membrane G and

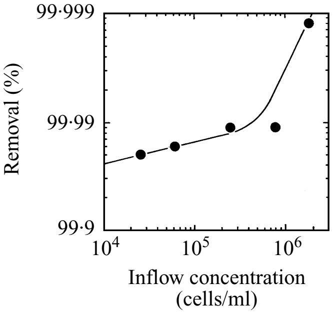

Fig. 6. Influence of the inflow concentration of airborne *E. coli* on the percentage removal by filtration through membrane E at a filtration rate of 63·7 cm/s.

E. coli as a test bacterium. Results are shown in Figure 5 (2). On average, 99·8 % of the bacteria were removed (2·7 $\log_{10}$-unit reduction in concentration) by the filtration. Differences in thickness, mean pore diameter, porosity, and particle diameter of the beads between membranes E and G were not great (Table 1). Figures 3 and 4 show a close resemblance of physical structure between these membranes. The difference in removal efficiency between these membranes can probably be attributed to differences in chemical structure, i.e. to the contribution of the pyridinium group contained in membrane E.

Removal of airborne bacteria by filtration using non-woven cloth coated with a pyridinium-type polymer

Results are shown in Figure 5 (3). On average, 94 % of the bacteria were removed (1·2 $\log_{10}$-unit reduction in concentration) by the filtration. When filtration using non-woven cloth not coated by the pyridinium-type polymer was performed in a similar manner, on average, 83 % of the bacteria were removed (0·8 $\log_{10}$-unit reduction in concentration) by the filtration (Fig. 5 (4)). Pore diameter of the cloth was about 540 μm.

Influence of the inflow concentration on bacterial removal efficiency

Removal of airborne bacteria by filtration through membrane E was investigated at low bacterial concentrations using *E. coli* as a test organism. The percentage removal reduced with decreasing inflow concentration (Fig. 6). However, a three $\log_{10}$-unit reduction in concentration was maintained when the inflow concentration of bacteria was less than 10 cells/ml. Filtration using membrane E appeared to be reliable even at low bacterial concentrations. Although 10 cells/ml is still a very high concentration for bacteria in air, and removal efficiency at much lower concentrations should be examined, such experiments were not performed. However, Figure 6 suggests results at a very low concentration of the bacteria are likely to be favourable.

Removal of *Staphylococcus aureus* and *Pseudomonas aeruginosa* by membrane filtration

Removal of airborne *S. aureus* by filtrations using membrane E and other related filter materials was carried out in a similar manner at a filtration rate of 63·7 cm/s. Results are summarized in Table 2. Filtration using membrane E showed complete removal, with *S. aureus* not detected in the filtrate. The removal percentage was estimated to be larger than 99·996 %.

Removal of airborne *P. aeruginosa* by filtration was performed in a similar manner at a filtration rate of 63·7 cm/s. Results are summarized in Table 3.

DISCUSSION

This study has demonstrated excellent removal of airborne bacteria by filtration using a composite microporous membrane made of cross-linked poly(*N*-benzyl-4-vinylpyridinium chloride), a functional polymer that showed strong affinity with bacterial cells, and reinforced by non-woven cloth. Membrane E was most suitable for this purpose among a variety of filter material developed. The excellent removal of airborne bacteria by filtration appeared to be attributable to efficient capture of bacterial cells on the surface of the pyridinium-type polymer. The large inner surface area of pores of the membrane probably enhanced efficiency of removal of airborne bacteria.

Filtration using membrane E was performed easily with insignificant pressure drop across the membrane, probably because the pore diameter and porosity of the membrane were sufficiently large. Conventional filter materials used for clean bench and other related facilities to remove airborne bacteria are designed to remove much smaller particles than the pore size, 14·5 μm, of membrane E. For example, HEPA filters are capable of removing particles larger than 0·2 μm. Although HEPA filters are fibrous in nature and the concept of pore size is not useful, the bacterial clearance capacity would be equivalent to membranes having a pore size of 0·2 μm. High removal efficiency with low filtration pressure would be important in practice.

Table 2. *Removal of airborne* Staphylococcus aureus *by filtration**

Filter material	Concentration (cells/ml) Inflow	Concentration (cells/ml) Effluent	Removal (%)
Composite membrane E	$2{\cdot}36 \times 10^4$	0	100
Composite membrane F	$2{\cdot}36 \times 10^4$	$4{\cdot}80 \times 10^1$	99·8
Coated non-woven cloth†	$2{\cdot}36 \times 10^4$	$1{\cdot}29 \times 10^3$	95
Non-coated non-woven cloth	$1{\cdot}49 \times 10^4$	$9{\cdot}95 \times 10^2$	93

* Filtration rate was 63·7 cm/s. Five samples of inflow and effluent air were withdrawn every time.

† Non-woven cloth coated with 40 mg/g of a 2:5 molar ratio copolymer of *N*-benzyl-4-vinylpyridinium chloride and styrene.

Table 3. *Removal of airborne* Pseudomonas aeruginosa *by filtration**

Filter material	Concentration (cells/ml) Inflow	Concentration (cells/ml) Effluent	Removal (%)
Composite membrane E	$1{\cdot}29 \times 10^5$	$3{\cdot}80 \times 10^0$	99·996
Composite membrane F	$1{\cdot}29 \times 10^5$	$4{\cdot}62 \times 10^1$	99·96
Coated non-woven cloth†	$1{\cdot}29 \times 10^5$	$2{\cdot}68 \times 10^2$	99·8
Non-coated non-woven cloth	$2{\cdot}48 \times 10^5$	$1{\cdot}07 \times 10^3$	99·5

* Filtration rate was 63·7 cm/s. Five samples of inflow and effluent air were withdrawn every time.

† Non-woven cloth coated with 40 mg/g of a 2:5 molar ratio copolymer of *N*-benzyl-4-vinylpyridinium chloride and styrene.

The pore size and the porosity of membrane F appeared to be too large for effective removal of airborne bacteria, probably due to insufficient contact of nebulized air with the inner surface of the membrane.

In previous applications, removal of airborne bacteria at very low concentrations would be required. The percentage removal by filtration using membrane E reduced with decreases of the inflow concentration of bacteria. However, the percentage removal of *E. coli* using one sheet of membrane E was still 99·9% (three $\log_{10}$-unit reduction in concentration) when the inflow concentration of the bacteria was less than 10 cells/ml (Fig. 6). Thus filtration using membrane E seemed to be reliable even at low bacterial concentrations.

Although a mechanical stirrer was used as a fan to prevent losses of bacteria in the air suspension by impingement on the surfaces, complete exclusion of these losses was difficult under the experimental conditions. The unusual increase in the removal efficiency at high concentration of bacteria (Fig. 6) may be attributed to a significant loss of bacteria by impingement on the surfaces under the experimental conditions employed.

It would be interesting to perform removal experiments using large volume air samples collected by impinger, but such experiments were impossible when the apparatus shown in Figure 1 was used. Further investigation is required. The aerosols produced by the apparatus described here will have very different properties to the great majority of bacteria-bearing particles occurring naturally in the air. The vehicles of bacteria often constitute the majority of the particles to be removed.

Since non-woven cloth coated with a pyridinium-type polymer was effective in removing waterborne bacteria [2], removal of airborne bacteria by filtration using non-woven cloth coated with 4·0 wt % of the copolymer of *N*-benzyl-4-vinylpyridinium chloride and styrene was performed using *E. coli* as a test bacterium. If the difference in removal efficiency between the composite membrane and the coated cloth was insignificant, the coated cloth would be more desirable than the composite membrane, because preparation of the coated cloth was much easier than the composite membrane. Differences in the removal efficiency between the coated cloth and the non-coated cloth could be attributed to a strong affinity between the pyridinium-type polymer and bacterial cells.

Differences in the removal efficiencies of membrane E and the coated non-woven cloth can be explained by the differences in effective surface areas of these filter materials. Mean pore diameters of membrane E and the coated non-woven cloth were 14·5 and 540 μm, respectively. As can be seen in Figure 3, membrane E consisted of a large number of small beads of pyridinium-type polymer and the surface area of the membrane would be very large. The coated non-woven cloth consisted essentially of piled fibres and the surface area of the cloth would be very small.

Excellent removal of airborne bacteria by filtration using membrane E seems to be based on efficient capture of bacterial cells on the surface of the pyridinium-type polymer. Affinity of the pyridinium-type polymer was especially strong for *S. aureus* among a variety of bacteria examined [3]. Thus composite microporous membranes made of the pyridinium-type polymer would be especially suitable for removing *S. aureus* from air, and filtration using membrane E could be expected to be effective for the removal of MRSA from hospital air. The present study was aimed at preliminary evaluation of the ability of the composite membrane to remove bacteria from air, and the experimental results are insufficient to determine accurately significant differences in the removal efficiency between bacterial species.

Based on these preliminary results, membrane E could be expected to be useful as an efficient filter material for removing airborne bacteria from hospital and other environments.

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