Removal of *Escherichia coli* from Water by Systems Based on Insoluble Polystyrene–Poly(ethylene Glycol)s, –Polyethylenimines, and –Polyethylenepolyamines Quaternized

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SYNOPSIS

Insoluble polymers adsorbing bacterial cells were prepared by reactions of chloromethylated, divinylbenzene crosslinked polystyrene (CMPS) beads with poly(ethylene glycol) 600 (PEG600), PEG monolaurate (PEGLE), polyethylenimines (PEIs, MW = ca. 300, 600, and 1200, referred to as PEI300, PEI600, and PEI1200, respectively), as well as ethylenediamine (ED) and tetraethylenepentamine (TEP) as polyethylenepolyamines. CMPS–ED and CMPS–TEP were further quaternized with 1-iodooctane (IO) and 1-iodododecane (IDD), respectively. These polymers were brought into contact with *Escherichia coli* by stirring in sterilized, distilled, and deionized water. Although CMPS–PEG and CMPS–PEGLE did not adsorb the cells, they caused a decrease in the number of viable cells. The decrease seemed to result from the bactericidal action of substances leached from the polymers. CMPS–PEI300, CMPS–PEI600, and CMPS–PEI1200, CMPS–ED–IO, and CMPS–TEP–IDD caused a decrease in the viable cell number by adsorption of the cells to their surfaces. It was observed with a scanning electron microscope that the cells were present on the surfaces of CMPS–PEI600 beads. The decrease coefficient for decrease in viable cell number of *E. coli* caused by the polymer increased with nitrogen content of the polymer. The adhesion of the cells to CMPS–PEI300, CMPS–PEI600, and CMPS–PEI1200, CMPS–ED–IO, and CMPS–TEP–IDD was due mainly to electrostatic interaction between them.

INTRODUCTION

Chlorine is used to disinfect and sterilize water for domestic supply. However, since chlorine reacts with organic substances in the water to yield halomethane analogues and carcinogens, its use should be avoided, as well as other disinfectants that lead to the defect of their residue. This defect can be solved by removal of microorganisms from the water with insoluble substances. Also, it is desirable from the viewpoint of purification of the water that viable and dead organisms are removed from it.

Quaternary ammonium salt groups, amines, alcohols, and cationic, anionic, and nonionic surfactants have antimicrobial activity towards bacteria and fungi.[1] Studies have been made on the antimicrobial activity of insoluble polymers[2] and adsorption of bacterial cells onto one such polymer,[3] antibacterial activity of soluble polymers,[4–6] and removal of bacterial cells by insoluble polymers.[7,8]

Although insoluble polystyrene into which were incorporated nonionic surfactants, amines, or alcohols should have antimicrobial activity, no studies apparently have been made on them. This study is concerned with the removal of *E. coli* cells from water by insoluble polymers derived from the reactions of chloromethylated, divinylbenzene crosslinked polystyrene beads (CMPS) with the compounds described above.

MATERIALS AND METHODS

Materials

CMPS obtained commercially (Polyscience Inc., Lot No. 34126) was 200–400 mesh (74–37 μm) beads,

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consisting of chloromethylstyrene 31.54 mol %, styrene 66.46 mol %, and divinylbenzene 2.00 mol %. CMPS was shaken and washed in methyl alcohol, very fine powder beads were removed by decantation, and the CMPS was filtered from the alcohol and dried.

Compounds used for reactions with CMPS are listed in Table I. These compounds were used without further purification. Solvents were used after dehydration and distillation.

Preparation of Polymers

CMPS beads react easily with alcohols and amines to yield insoluble polystyrene-type polymers. Reactions of CMPS with PEG600 or PEGLE[9] and those with PEI300, PEI600, or PEI1200, and ED or TEP[10] were carried out in the same manner as in the previous studies.

The reaction of CMPS–TEP with IDD was conducted as follows: A mixture of CMPS–TEP (4.10 g), dioxane (10 mL), and IDD (8.65 g) was stirred at 95–100°C for 24 h. The mixture was filtered and the polymer thus obtained was washed successively on the filter with dioxane, methyl alcohol, and boiling water and dried. Yield 5.50 g.

All polymers were then washed with methyl alcohol for 3 days using a Soxhlet extractor. After stirring all the polymers in 50 mL of boiling distilled water for 24 h, they were collected by filtration. This procedure was repeated five more times to remove PEG, PEGLE, PEI, ED, TEP, IO, and IDD groups from the polymers.

Organism and Growth Conditions

The bacterium used was *E. coli* IFO 12734, which was obtained commercially from the Institute for Fermentation, Osaka.

One loopful of *E. coli* IFO 12734 was inoculated into 10 mL of nutrient broth (Difco) and cultured at 37°C for 18 h. After the cells in 6.5 mL of the cultured cell suspension were collected by centrifugation at 2200 × *g* for 12 min in a centrifuge refrigerated below 4°C, washed twice with 6.5 mL of distilled, deionized, and sterilized water (hereafter referred to as sterilized water), they were again suspended in 6.5 mL of fresh sterilized water.

Contacts of Polymers, PEG600, or PEI600 with Cells

One-tenth to 0.250 g of each polymer was placed in a round-bottomed 50-mL flask, and then 18 or 19 mL of water were poured into it. The polymer was completely wetted, and then stirred at about 300 rpm using a magnetic stirrer. Two or 1 mL of a cell suspension (10^{8-9} cells/mL) prepared as described above were added into the flask kept in a thermostatted bath at 37°C, thus bringing the total volume to 20 mL. The polymer was brought into contact with the cells by magnetic stirring.

Contact of PEG600 or PEI600 with bacterial cells was carried out as follows: (19 or 18 − x) mL of sterilized water were added to a round-bottomed flask containing x mL of an aqueous solution of PEG600 or PEI600 1000 μg/mL. Then, 1 or 2 mL of a cell suspension (10^{8-9} cells/mL) were added to the flask (total volume = 20 mL, the concentration

Table I Compounds, Reaction Products, and Their Abbreviation

	Compound (Source)[a]	Abbreviation of Compound	Abbreviation of Product
CMPS	Poly(ethylene glycol) 600 (Wako Junyaku Co.)	PEG600	CMPS–PEG600
	Poly(ethylene glycol) monolaurate (Tokyo Kasei Co.)	PEGLE	CMPS–PEGLE
	Polyethylenimine MW ca. 300 (Nippon Shokubai Kagaku Kogyo Co.)	PEI300	CMPS–PEI300
	Polyethylenimine MW ca. 600 (Nippon Shokubai Kagaku Kogyo Co.)	PEI600	CMPS–PEI600
	Polyethylenimine MW ca. 1200 (Nippon Shokubai Kagaku Kogyo Co.)	PEI1200	CMPS–PEI1200
	Ethylenediamine (Tokyo Kasei Co.)	ED	CMPS–ED
	Tetraethylenepentamine (Tokyo Kasei Co.)	TEP	CMPS–TEP
CMPS–ED	1-Iodooctane (Alfa Products)	IO	CMPS–ED–IO
CMPS–TEP	1-Iodododecane (Alfa Products)	IDD	CMPS–TEP–IDD

[a] These compounds were obtained commercially or supplied from respective companies.

$= 50 \cdot x\ \mu g/mL$). The suspension thus prepared was stirred as described above.

Measurement of Viable Cell Number in the Contact Suspension

Before sampling, the stirring of the contact suspension was stopped, and the suspension was allowed to stand for 2 min until the polymer beads settled. One-tenth milliliter of the contact suspension was pipetted out from the flask at known time intervals, inoculated into 9.9 mL of sterilized water, and serially diluted with the water. One-tenth milliliter of the diluted suspension was spread on an agar plate made of nutrient agar (Difco). The plate was kept at 37°C for 15–20 h, and the number of viable cells in the contact suspension was calculated from that of the colonies formed on the plate.

Scanning Electron Microscopy

CMPS–PEI600 beads, which were brought into contact with viable *E. coli* cells for 4 h, were collected on a no. 3 glass filter, and rinsed on the filter three times with 10 mL of sterilized water. The rinsed beads were placed in 2% aqueous solution of glutaraldehyde (Wako Junyaku Co.). Subsequent treatments (fixation with osmium oxide solution, dehydration with a graded ethyl alcohol and acetone series, and others)[3] and photographing with a scanning electron microscope Model S-800 (Hitachi Co.) were performed by the Toray Research Co.

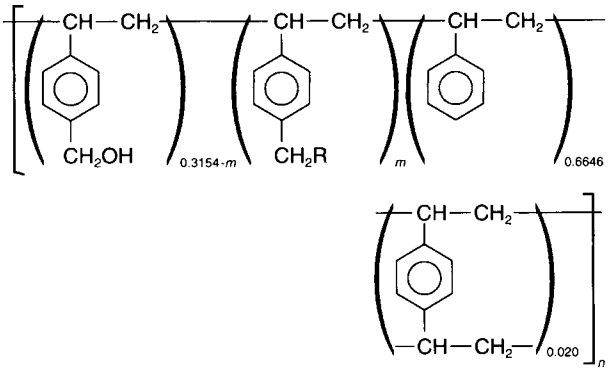
Measurement of Total Organic Carbon Content

While the polymers were in contact with the cell suspensions, it was found by measurement with a Hitachi spectrophotometer Model 220 A that certain substances were leached from the polymers into the suspension. Thus, total organic carbon content (TOC) of substances which were leached from polymers into water was determined as follows: One-quarter gram of a polymer was stirred in sterilized water for 4 h, following the contact with cells. The polymer was immediately filtered using a no. 3 glass filter. The amount of TOC in this filtrate was measured using a total organic carbon meter Model TOC-10 B (Shimadzu Corp.).

RESULTS

Reactions of CMPS with PEG or PEI

Table II lists the results of elemental analyses and the molar number of reacted groups calculated[10] on the basis of the results of elemental analysis. The prepared polymers are presumed to have the following probable chemical structures:



m = the mole number of chloromethylstyrene unit which reacted with PEG600, PEGLE, PEI300, PEI600, PEI1200, ED, or TEP. All or most of the unreacted chloromethyl groups were hydrolyzed during the reaction and the subsequent treatments to yield hydroxymethyl groups.

CMPS–PEG600: R = —$O(CH_2CH_2O)_{13.54}H$, $m = 0.167$

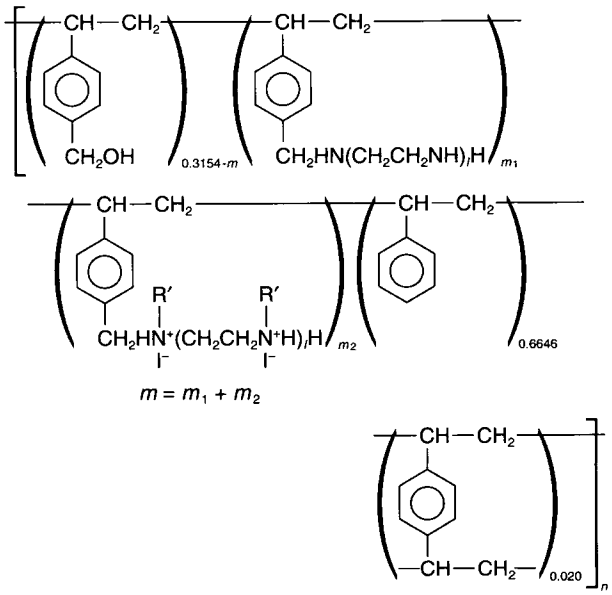
CMPS–PEGLE: R = —$O(CH_2CH_2O)_{10}(CH_2)_{11}CH_3$ $m = 0.160$

CMPS–PEI300: R = —PEI300 group, $m = 0.130$

CMPS–PEI600: R = —PEI600 group, $m = 0.120$

CMPS–PEI1200: R = —PEI1200 group, $m = 0.032$

Scheme 1 Probable chemical structure of CMPS–PEG600, CMPS–PEGLE, CMPS–PEI300, CMPS–PEI600, and CMPS–PEI1200



$m = m_1 + m_2$

CMPS–ED–IO: $l = 1$, $R' = (CH_2)_7CH_3$, $m = 0.225$

CMPS–TEP–IDD: $l = 4$, $R' = (CH_2)_{11}CH_3$, $m = 0.180$

Scheme 2 Probable chemical structures of CMPS–ED–IO and CMPS–TEP–IDD

In the reactions of CMPS–ED with IO and CMPS–TEP with IDD, only a part (m_2) in amino groups (m) were quaternized.

Contact of Polymers with *E. coli* Cells in Water and Decrease Coefficient

During stirring of the cells in sterilized water at 37°C without CMPS–PEI300, the viable cell number decreased hardly at all during contact for 4 h. When the cells were stirred with the polymer in water, the viable cell number decreased (Fig. 1). Contact of CMPS–PEI300 with viable cells was carried out with various initial cell numbers and amounts of CMPS–PEI300. For contact of CMPS–PEI300 0.100 g with an initial viable cell number of 5.2×10^7 cells/mL, the viable cell number decreased to 9×10^6 and 4×10^6 cells/mL after contact for 3 and 4 h, respectively. This shows that 0.1 g of the polymer adsorbed 9.6×10^8 viable cells [$= (52 - 4) \times 10^6$ (cells/mL) $\times$ 20 (mL)] after 4 h. More cells may be adsorbed with the contact for longer time.

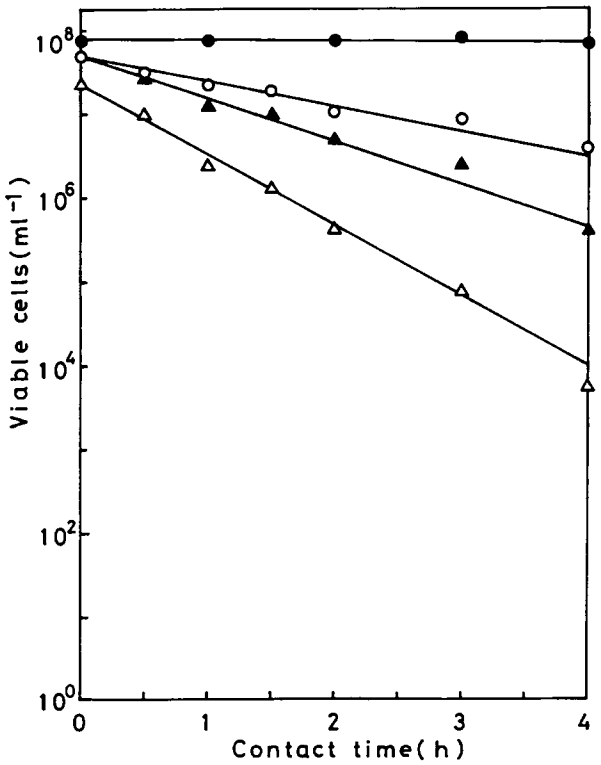


Figure 1 Decrease in viable cell number with the lapse of time in contact of *E. coli* cells with CMPS–PEI300 in sterilized, distilled, and deionized water (sterilized water) at 37°C. CMPS–PEI300 (g): (●) 0; (○) 0.100; (▲) 0.150; (△) 0.250.

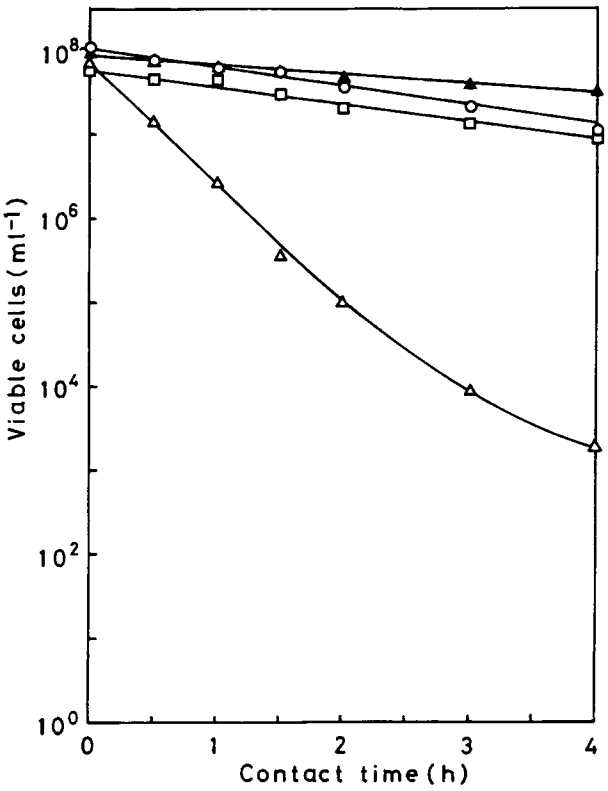


Figure 2 Decrease in viable cell number with the lapse of time in contact of *E. coli* cells with polymers in sterilized water: (○) CMPS–PEG600 0.100 g; (□) CMPS–TEP–IDD 0.100 g; (△) CMPS–PEI600 0.100 g; (▲) CMPS–ED–IO 0.100 g.

Contacts of the cells with other polymers were carried out, and the decrease behaviors of viable cell number are shown in Figure 2. All these polymers have functions which make the viable cell number decrease. This ability varied according to the group introduced in CMPS.

In order to compare quantitatively these functions, the following was considered. In the early stage of contact, a linear relation was observed between the logarithm of viable cell number and contact time. This fact indicated that the decrease behavior of a viable cell number was a first-order process similar to Kao et al.'s,[11] Isquith and McCollum's,[12] and Kawabata et al.'s.[7] Thus the decrease coefficient of viable cell number (D) was defined as eq. (1) and calculated:

$$D = \frac{V}{W \cdot t} \log \frac{N_0}{N_t} \qquad (1)$$

where V is the volume (mL) of viable cell suspension, W the dry weight (g) of the polymer, t the

contact time (h), N_0 the initial viable cell number, and N_t the viable cell number (cells/mL) at contact time t.

Table III lists D. Even if the amount of a polymer and a initial cell number are different, the D values for *E. coli* by each polymer were similar in the range of the experimental conditions described in Table III. Since CMPS beads were difficult to wet with water and the greater part of them did not sink, the D could not be determined.

The D values for *E. coli* by the polymers were in the order of CMPS–PEI600 > CMPS–PEI300 > CMPS–PEI1200 ≥ CMPS–PEG600 > CMPS–TEP–IDD > CMPS–ED–IO > CMPS–PEGLE. Except for CMPS–PEG600 and CMPS–PEGLE, D is greater in a polymer with more nitrogen content than one with less nitrogen content (Table III).

Adsorption of Viable and Dead Cells by Polymers

When CMPS–PEI300, CMPS–PEI600, CMPS–PEI1200, CMPS–ED–IO, or CMPS–TEP–IDD were mixed with a cell suspension, the optical density at 660 nm (OD_{660}) decreased after contact for 4 h (Table III).

By contact with CMPS–PEG600 or CMPS–PEGLE having nonionic group, the viable cell number decreased with time. However, OD_{660} scarcely decreased even after contact for 4 h. Therefore, none of the cells adhere to CMPS–PEG600 and CMPS–PEGLE. Bacterial cells were observed on the surfaces of CMPS–PEI600 beads with a scanning electron microscopy (Fig. 3), but not on those of CMPS–PEG600 (Fig. 4).

An examination was made to determine whether or not CMPS–PEI600 adsorbs *E. coli* dead cells from water. The contact study was performed using *E. coli* cells which were heated at 80°C for 30 min. The OD_{660} of the cell suspension was 0.101 before the contact, and it decreased to 0.054 after the contact with CMPS–PEI600 0.100 g for 4 h. This demonstrates that CMPS–PEI600 beads also adsorb dead cells from water.

Effects of PEG600 and PEI600 on Cells

During the stirring of each polymer in a cell suspension, PEG, PEGLE, PEI, ED, TEP, or other substances leached from each polymer may cause a decrease in the viable cell number. Thus, the effect

Table II Reaction Conditions and the Elementary Analysis Results of the Polymers Prepared

Polymers	CMPS	Reactant (g)	Solvent (mL)	React. Temp (°C)	React. Time (h)	Yield (g)	Reacted CH_2Cl Groups (mol %)	Combined Groups (mmol/g CMPS)	H (%)		C (%)		N (%)		Cl[b] (%)	I (%)	
									f	*c*	*f*	*c*	*f*	*c*	*f*	*f*	*c*
CMPS–PEG600	3.00	9.6	Diox. 25	95	30	5.18	16.7	1.39	8.52	8.33	72.57	72.57	0.00	—	−		
CMPS–PEGLE	3.00	10.0	Diox. 25	95	168	4.34	16.0	1.33	8.46	8.90	74.63	75.59	0.11	—	+		
CMPS–PEI300	6.00	9.5	DMF 40	100	120	8.38	13.0	1.08	8.35	8.29	74.36	78.37	8.99	8.94	4.24		
CMPS–PEI600	6.00	9.5	D, B[c] 40	95	192	9.08	12.0	1.00	8.87	9.28	74.29	75.75	12.97	13.29	0.00		
CMPS–PEI1200	5.00	15.8	DMF 40	100	168	6.27	3.2	0.27	8.39	8.50	79.76	77.66	8.62	8.40	+		
CMPS-ED	3.00	2.4	DMF 20	100	192	3.44	22.5	1.58	7.39	7.99	90.08	84.10	4.97	5.03	−		
CMPS–TEP	3.00	4.5	DMF 20	100	160	4.12	18.0	1.50	8.08	8.42	74.31	81.15	8.43	8.70	+		
CMPS-ED–IO	3.00[d]	5.7[e]	Diox. 15	95	96	3.55	6.0[h]		7.37	8.06	79.53	80.76	4.53	4.52	0.00	5.45	5.15
CMPS–TEP-IDD	4.00[f]	8.6[g]	Diox. 10	95	28	5.05	18.0[i]		7.66	8.62	71.16	72.41	6.49	6.36	0.00	11.85	11.52

[a] Diox. = dioxane, B = benzene.
[b] + shows Cl was detected by the Beilstein test. − shows Cl was not detected.
[c] Dioxane 20 mL + benzene 20 mL.
[d] CMPS-ED (ethylenediamine).
[e] 1-Iodooctane.
[f] CMPS–TEP (tetraethylenepentamine).
[g] 1-Iodododecane.
[h] Mol % IO reacted with CMPS-ED.
[i] Mol % IDD reacted with CMPS–TEP.

Table III Decrease Coefficent (D)[a] of Viable Cell Numbers from Water by Contact of *E. coli* with Each Polymer

Polymer	N (%)	Combined[b] Groups (mmol/g polymer)	Added Polymer (g)	Initial Viable Cells (cells/mL)	D^a (mL/g h)	OD_{660} of Cell Suspension Before Contact	OD_{660} of Cell Suspension After Contact
CMPS–PEG600		0.790	0.100	1.2×10^8	50	0.058	0.066
			0.250	9.5×10^{6} [e]	50	0.107	0.128
			0.250	1.4×10^{8} [e]	44	0.086	0.090
CMPS–PEGLE		0.747	0.250	6.1×10^7	14	0.040	0.048
			0.250	4.6×10^7	12	0.040	0.047
CMPS–PEI300	8.99	0.843	0.100	5.2×10^7	65	0.048	0.014
			0.150	5.0×10^7	70	0.049	0.001
			0.150	1.2×10^8	74	0.072	0.002
			0.150	6.2×10^7	68	0.039	0.001
			0.250	6.2×10^7	88	0.043	0.001
			0.250	1.0×10^7	66	0.048	0.001
CMPS–PEI600	12.97	0.640	0.100	7.1×10^7	290	0.053	0.024
			0.100	8.2×10^7	260	0.063	0.034
			0.100	6.0×10^7	250	0.054	0.021
			0.250	1.0×10^{8} [e]	270	0.120	0.032
			0.250	3.5×10^{8} [e]	260		
CMPS–PEI1200	8.62	0.210	0.100	4.3×10^7	50	0.046	0.013
			0.250	6.5×10^7	46	0.035	0.001
CMPS–ED–IO	4.53	0.434[c]	0.100	9.0×10^7	25	0.059	0.052
			0.250	6.2×10^7	27	0.041	0.004
CMPS–TEP–IDD	6.49	0.908[d]	0.100	5.8×10^7	43	0.052	0.018
			0.250	1.5×10^{8} [e]	35	0.116	0.003
			0.250	4.3×10^7	37	0.040	0.001

[a] Determined at 37°C by the contact of the insoluble polymer 0.100–0.250 g with 20 mL of viable *E. coli* cell suspension.
[b] Mole number of PEG600, PEGLE, PEI300, PEI600, or PEI1200 groups contained in 1 g of each polymer.
[c] Mole number of IO groups contained in 1 g of the polymer.
[d] Mole number of IDD groups contained in 1 g of the polymer.
[e] The added amount of a cell suspension: 2 mL; the others: 1 mL.

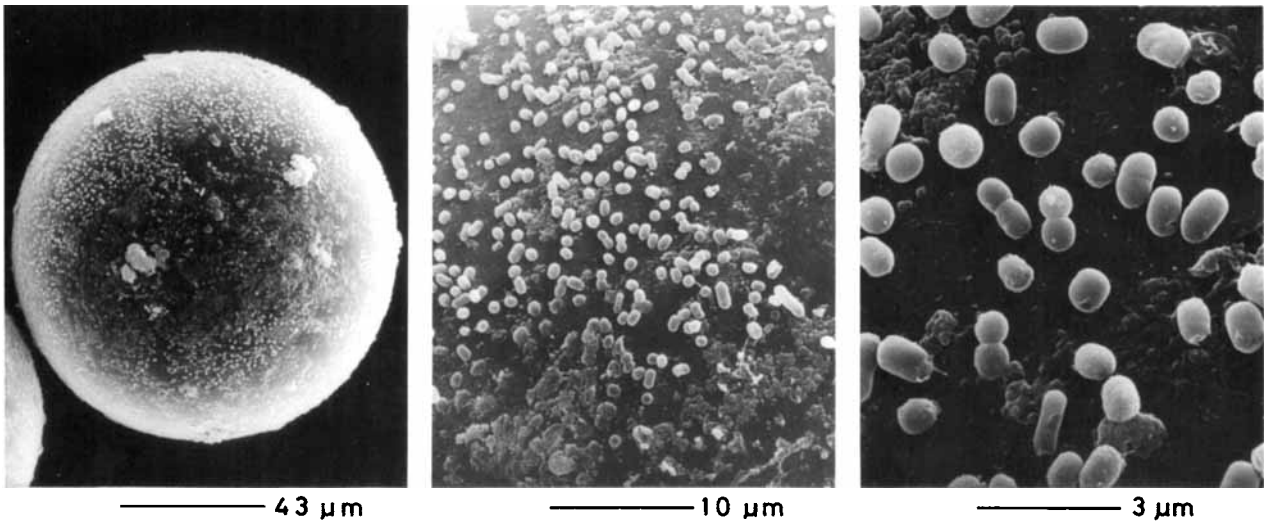


Figure 3 Scanning electron micrographs of the surface of a CMPS–PEI600 bead which was brought into contact with a *E. coli* cell suspension.

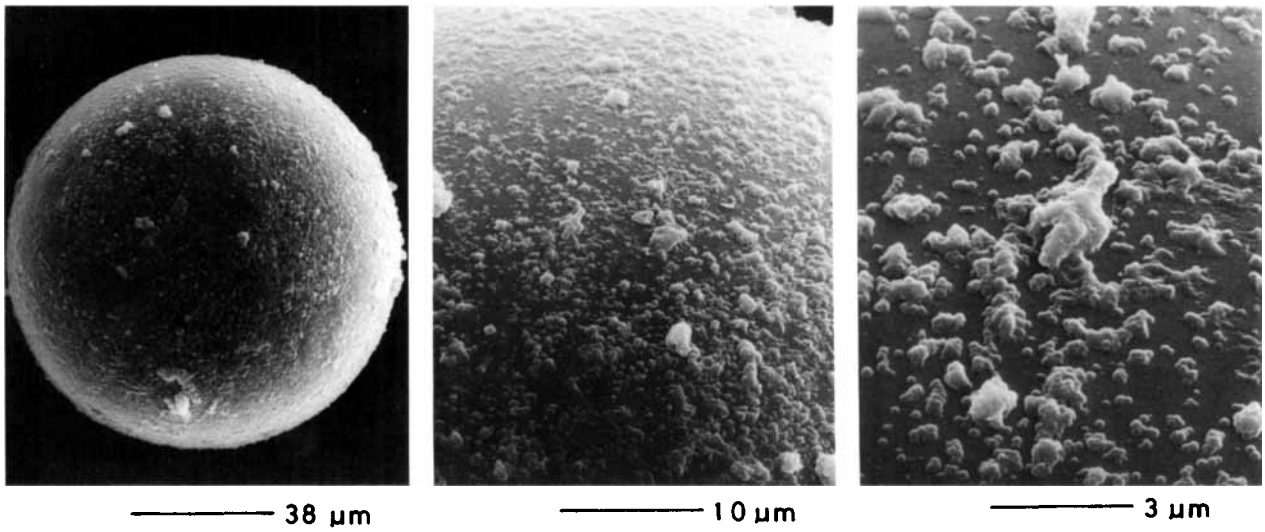


Figure 4 Scanning electron micrographs of the surface of a CMPS–PEG600 bead which was brought into contact with a *E. coli* cell suspension.

of PEG600 on *E. coli* viable cells was examined by stirring the cells in its aqueous solution. TOC of substances, which was leached from 0.250 g of CMPS–PEG600 into water, was 25 μg/ml, corresponding to about 50 μg/ml as the concentration of PEG600.

The bactericidal activity of PEG600 was measured by stirring the cells (around 10^8 cells/mL) in sterilized water containing PEG600 50–100 μg/mL (Fig. 5). It was small in the cases of PEG600 concentration below 100 μg/mL. Assuming that PEG600 of 100 μg/mL was leached from 0.100 g of CMPS–PEG600, the *D* value should be less than 16.

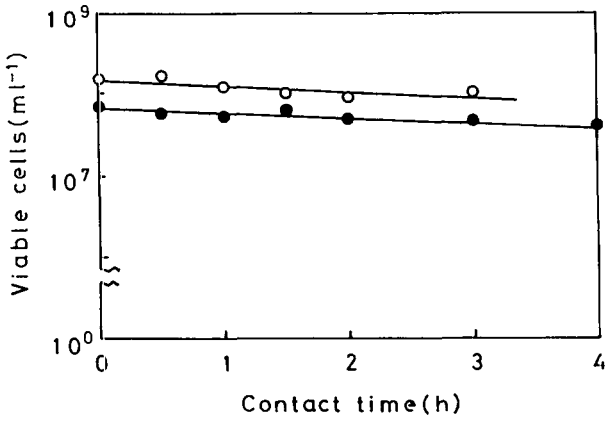


Figure 5 Decrease in viable cell number with the lapse of time in contact of *E. coli* cells with PEG600 in sterilized water. Added volume of a cell suspension (10^{8-9} cells/mL) prepared beforehand: 2 mL. PEG600 (μg/mL): (○) 50; (●) 100.

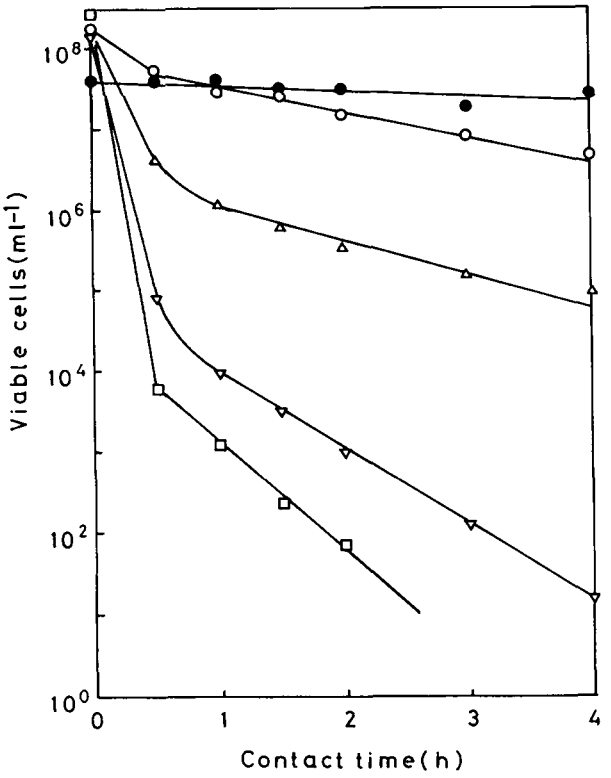


Figure 6 Decrease in viable cell number with the lapse of time in contact of *E. coli* cells with PEI600 in sterilized water. Added volume of a cell suspension (10^{8-9} cells/mL) prepared beforehand: 2 mL. PEI600 (μg/mL): (○) 5; (△) 10; (▽) 25; (□) 50; (●) TOC 20 μg/mL. The last contact cell suspension was prepared in the following way: After 0.250 g of CMPS–PEI600 was stirred similarly to the contact with cells, the polymer was filtered, and 1 mL of a cell suspension was added to 19 mL of the filtrate.

TOC of substances, which was leached from 0.250 g of CMPS–PEI600 into water, was 21 μg/mL, corresponding to PEI600 concentration of about 40 μg/mL. The decrease in viable cell number was examined by stirring the cells in sterilized water containing PEI600 5–50 μg/mL (Fig. 6). The degree of decrease in the viable cell number increased with an increase in the concentration of PEI600. After 0.5–1 h from the start of contact, linear relations were observed between logarithm of the viable cell number and contact time. The bactericidal activity of the filtrate on *E. coli* cells was found to be less than that for PEI600 5 μg/mL (Fig. 6).

Since the total amount of substances leached from CMPS–ED–IO or CMPS–TEP–IDD was less than 20 μg/mL, its effect on viable cells appears small as also the case for CMPS–PEI600.

Viability of Cells on Polymer Beads

After the beads of CMPS–PEI600, previously in contact with an *E. coli* cell suspension, were rinsed with sterilized water, cell proliferation was observed at the time they were cultured by being spread on an agar plate and placed in an aqueous solution of nutrient broth. The cells on the surfaces of CMPS–PEI600 beads were found to be adsorbed alive, as were also those on poly(4-vinylpyridinium halide).[7]

DISCUSSION

Adhesion of Cells to Polymers

CMPS–PEI600 adsorbed both viable and dead cells from water. It has been reported that poly(vinylpyridinium halid) adsorbs not only *E. coli* viable, but also dead cells.[7]

It is proposed that the adhesion of bacterial cells to solid surfaces is caused by electrostatic, hydrophobic interaction, and other forces of attraction (hydrogen bonding, van der Waals forces, etc.).[13–16] Quaternized nitrogen atoms in CMPS–ED–IO and CMPS–TEP–IDD behave as cations in water. Partially acylated polyethylenimine is protonated to a considerable extent in water of pH 7.[17] Accordingly, nitrogen atoms in CMPS–PEI300, CMPS–PEI600, CMPS–PEI1200, and those not quaternized in CMPS–ED–IO and CMPS–TEP–IDD are partially protonated in water (pH 7) to yield cationic nitrogen atoms.

$$—NH— + H_2O \rightleftharpoons —N^+H_2— + OH^-$$

Bacterial cells have predominantly negative charges at physiological pH.[16] Also, Ikeda et al. state that a bacterial cell surface is usually negatively charged from its susceptibility of electrophoresis.[5] Therefore, it may be concluded that the bacterial cells adhere to a polymer mainly by electrostatic interaction during contact of CMPS–PEI300, CMPS–PEI600, CMPS–PEI1200, CMPS–ED–IO, or CMPS–TEP–IDD with the *E. coli* cell suspension. This is supported by the fact that *D* increases with the nitrogen content in CMPS–PEI300, CMPS–PEI600, CMPS–PEI1200, CMPS–ED–IO, and CMPS–TEP–IDD, showing the factor for this adhesion to be predominantly electrostatic interaction. It has been reported that the adhesion of bacterial cells to poly(lauryl-4-vinylpyridinium-*co*-divinylbenzene iodide) is due mainly to electrostatic interaction.[3]

After contact of polymers with cells, scanning electron microscopy was not performed on the surfaces of the polymers except for CMPS–PEI600 and CMPS–PEG600. However, judging from the differences in OD_{660} before and after the contact with cells, it can be presumed that *E. coli* viable cells also adhere to CMPS–PEI300, CMPS–PEI1200, CMPS–ED–IO, and CMPS–TEP–IDD, but not to CMPS–PEGLE.

The *D* value for *E. coli* by CMPS–PEI600 was 270 on the average and the removal coefficient (*R*) [Kawabata et al. defined the removal coefficient as eq. (1) based on the initial rate of decrease of viable cell counts[7]] for *E. coli* by poly(*N*-benzyl-4-vinylpyridinium bromide) was 4.3[7] and 7.1.[8] The *D* (270) was determined in sterilized water, and the *R* (4.3 and 7.1) in sterilized physiological saline. Since the experimental conditions differed, the values could not be compared. A comparison of both will be described in the subsequent paper.

Hydrophilicity of Polymers

The *D* values for *E. coli* by CMPS–PEI300, CMPS–PEI600, and CMPS–PEI1200, which were easy to wet, were greater than CMPS–ED–IO and CMPS–TEP–IDD, which were difficult to wet.

The hydrophobic portions of these polymers are styrene, vinylbenzyl, and divinylbenzene groups, and the hydrophilic portions are PEG, PEGLE, PEI, ED, and TEP groups. CMPS–PEGLE is more difficult to wet than CMPS–PEG due to bonding of dodecyl group to PEG. CMPS–ED–IO and CMPS–TEP–IDD are more difficult to wet than CMPS–ED and CMPS–TEP due to bonding of IO and IDD, re-

spectively. The *D* value for *E. coli* by CMPS–ED–IO with less IO groups is less than the *D* by CMPS–TEP–IDD with more IDD groups. This is attributable to the fact that CMPS–TEP–IDD has a higher nitrogen content than CMPS–ED–IO.

In these polymer systems, a factor such as hydrophobic interaction appears not to significantly contribute to the adhesion of *E. coli* viable cells to CMPS–PEI300, CMPS–PEI600, CMPS–PEI1200, CMPS–ED–IO, and CMPS–TEP–IDD. Kawabata et al.[7] reported that hydrophilicity of the polymer matrix used in their study enhanced the affinity of the polymer for bacterial cells. This tendency was also observed in this study. In the present polymer systems, a proportion of hydrophilic to hydrophobic portions seems to contribute to adsorption of cells.

Bactericidal Activity of Substances Leached from Polymers

Following contact of CMPS–PEG600 with a cell suspension, OD_{660} increased somewhat, and scanning electron microscopy showed that cells were not present on the surface of CMPS–PEG600 (Fig. 4). The *D* value for *E. coli* by CMPS–PEG600, however, was about 50 (mL/g h). Apparently the cells were killed by PEG600 and/or other substances leached from the polymer beads during contact.

CMPS–PEGLE also seems not to adsorb cells as well as CMPS–PEG600. The *D* values for *E. coli* by CMPS–PEGLE is small, from 12 to 14 (mL/g h). It has been reported that when viable *E. coli, Staphylococus aureus, Saccharomyces cerevisiae,* or *Aerobacter aerogenes* cells are placed in an aqueous solution of PEGLE 1000 μg/mL followed by stirring for 5 min, PEGLE shows no bactericidal activity.[18] The decrease in viable cell number by CMPS–PEGLE appears due to substances leached from the polymer during contact with cells.

PEI600 caused the number of viable *E. coli* cells to decrease greatly. When CMPS–PEI600 beads are stirred in water, substances other than PEI600 may leach from them. The greater part of 21 μg/mL may be other substances besides PEI600. It appears that the viable cell number did not decrease significantly for this reason. This is yet to be confirmed.

In any case, since the decrease in the number of viable *E. coli* cells by the bactericidal activity of substances leached from CMPS–PEI600 is small compared with that by the adsorption of cells onto the polymer, the effect of the substances can be neglected.

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