10. Biological System (2)

10.1 Capturing of *Staphylococcus aureus* onto an Interface Containing Graft Chains

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Abstract—A microbial-cell-capturing material was prepared by radiation-induced grafting of glycidyl methacrylate onto a polyethylene-based fiber before the introduction of diethylamine. The prepared fiber was tested against a *Staphylococcus aureus* and *Escherichia coli* solution. The results showed that the grafted-type fiber had a capturing rate constant 1000-fold higher than the commercial crosslinked-type bead for *S. aureus* and that an activation energy of 39 kJ/mol was obtained for the microbial-cell-capturing action.

INTRODUCTION

For water purification, although chlorine has been widely introduced after the discovery of its antibacterial activity, hazardous levels of trihalomethane analogues and carcinogens are produced in chlorinated water containing organic materials. As a result, some researchers have been searching for an alternative water disinfectant such as an insoluble polymeric substance with antimicrobial activity in order to avoid the problem of residual toxicity of soluble chlorine (Kawabata, 1983; Tashiro, 1991).

Usually, most of the insoluble antimicrobial materials prepared were derived from the copolymer beads crosslinked with divinylbenzene, where the functional groups were immobilized directly onto the polymeric support. However, a study on the interaction of microbial cells with a graft-chain-containing polymer has not yet been reported. The functional groups of this polymer are not immobilized directly onto the polymeric support but the grafted polymer branches. These graft chains provide the microbial cell a flexible network for interaction. Tsuneda et al. (1994) reported that the sulfonic-acid(SO_3H)-group-containing polymer chains grafted on a polyethylene-based membrane provided a three-dimensional adsorption site for lysozyme. Mizota et al. (1994) reported that a 10-fold higher catalytic activity of the SO_3H-group anchored to the flexible grafted polymer chains can be obtained for the hydrolysis of sucrose as compared to a commercial SO_3H-type crosslinked resin. These kinds of functional materials can be easily prepared by radiation-induced graft polymerization.

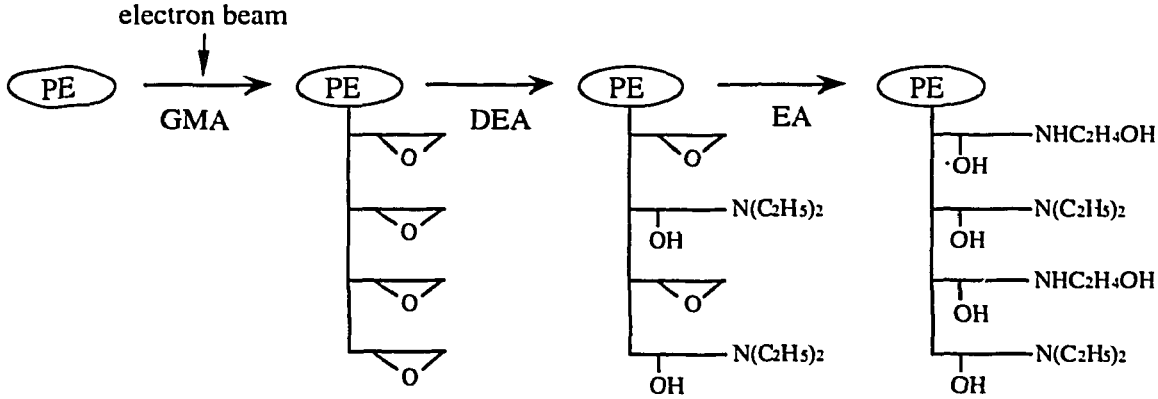
The radiation-induced graft polymerization (RIGP) has enabled the preparation of a novel polymeric material containing functional moieties on the branches grafted onto a variety of trunk polymers such as films, fibers, nonwoven cloth and hollow fibers (Sugo, 1989; Sugiyama, 1993). To date, we have developed various functional materials by applying RIGP, for instance, a chelating porous hollow-fiber membrane for the removal of metals from ultrapure water (Konishi, 1992), a urea-permeable anion-exchange membrane for the regeneration of dialysate in the peritoneal dialysis system (Lee, 1993), and a cation-exchange membrane for the separation of protein from biological fluids (Tsuneda, 1994).

Our previous paper (Lee, 1994) described that a grafted-type diethylamino-group-containing fiber had an adsorption rate constant of *Staphylococcus aureus* 1000-fold higher than the commercial crosslinked beads. Hence, the objectives of this study were threefold: (1) to propose a method for preparing an insoluble microbial-cell-capturing material which has a three-dimensional footing site by RIGP, (2) to study the influence of two coexisting functional groups, i.e., an epoxy group and an ethanolamino group, on the capturing of microbial cells, and (3) to measure the activation energy of the microbial cell-graft chain interactions in order to obtain some information on the mode of the microbial-cell-capturing action.

EXPERIMENTAL

Preparation of tertiary-amino-group-containing material

A commercial available polyethylene (PE) membrane of a hollow-fiber form (Asahi Chemical Industry Co., Ltd., Japan) was used as the trunk polymer for grafting. The inner and outer diameters of the hollow fiber are 1.9 mm and 3.2 mm, respectively, with a porosity of 70%. Figure 1 shows the chemical structure of the microbial-cell-capturing material prepared. A vinyl monomer, glycidyl methacrylate (GMA, $CH_2=CCH_3COOCH_2CHOCH_2$), was grafted onto the PE fiber by applying the RIGP method. The



PE: polyethylene GMA: glycidyl methacrylate DEA: diethylamine
EO: epoxide group EA: 2-aminoethanol

Fig 1. Chemical structure of the microbial-cell-capturing material prepared.

degree of GMA grafting was set to about 110%. The GMA-containing fiber was reacted with diethylamine (DEA, $HN(C_2H_5)_2$) for the introduction of cationic graft chains. The resulting fiber will be referred to as a DEA-EO fiber. The reaction conditions for radiation-induced grafting of GMA and subsequent introduction of tertiary amino groups (DEA) are described elsewhere (Lee, 1993). On the other hand, the residual epoxy group of DEA-EO fiber was hydrophilized by ethanol amine (EA, $H_2NCH_2CH_2OH$) and the resulting fiber will be referred to as a DEA-EA fiber.

For comparison, commercial GMA beads (FP-4000; Organo Co., Japan) crosslinked with ethylene glycol dimethacrylate were obtained and the same tertiary amino groups, DEA groups, were also introduced according to the same reaction conditions. The resulting beads will be referred to as DEA beads.

Bacterium culture and growth conditions

Escherichia coli IFO 3301 and *Staphylococcus aureus* IFO 12732 obtained commercially from the Institute for Fermentation, Osaka, were used as model microorganisms for the microbial-cell-capturing studies. One loopful of the bacteria was inoculated into a 10 mL of rehydration fluid (polypeptone 1.0%, yeast extract 0.2%, $MgSO_4 \cdot 7H_2O$ 0.1%, pH 7.0) and cultured at 32 °C for 18-24 h in a test-tube shaker at 100 strokes/min. After the cells in the cultured cell suspension were collected by centrifugation at 5600 × g for 15 min in a centrifuge refrigerated below 4 °C, and washed twice with 10 mL of distilled, deinoized, and sterilized water, they were again suspended in 10 mL of fresh sterilized water. The cells in the stationary state were serially diluted in sterilized water to the desired cell concentration (about 10^{5-6} cells/mL) before contact with the prepared fibers.

Batch experiment for capturing of microbial cell by DEA-EO and DEA-EA fibers, DEA beads

Forty milliliter of the cell suspension prepared were added into a 100 mL flask. The DEA beads, DEA-EO and DEA-EA fibers were brought into contact with the cells by shaking at 130 rpm at 25 °C.

One-tenth milliliter of the contact suspension was pipetted out from the flask at specific time intervals, inoculated into a 9.9 mL of sterilized water, and serially diluted with sterilized water. One-tenth milliliter of the diluted suspension was spread on an agar plate made of growth media. The plate was incubated at 37 °C for 18-20 h, and the number of viable cells in the contact suspension was calculated from that of the colony-forming units per milliliter (CFU/mL) on the plate.

Activation energy of microbial-cell-capturing action

The microbial-cell-capturing action of the DEA-EO (89% of DEA conversion) fiber was tested in the same batch mode at a temperature ranging from 15 to 37 °C. The values of the microbial-cell-capturing rate constant were determined from the slopes of the logarithm of viable cells vs contact time plots, and then the activation energy of the action was obtainable from the Arrhenius plot.

RESULTS AND DISCUSSION

Conversion of tertiary amino group (DEA) for the

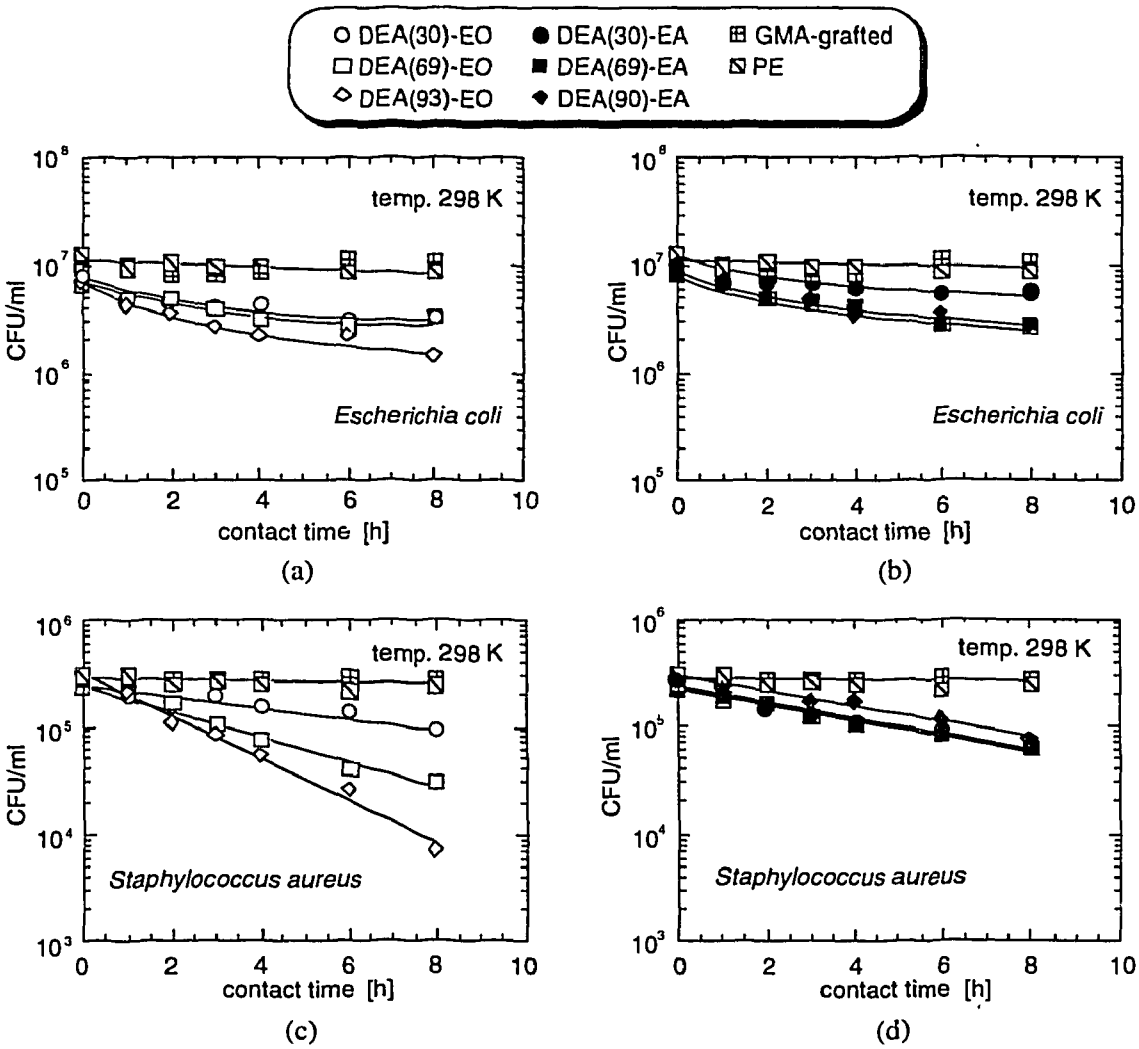


Fig 2. Effect of DEA-EO and DEA-EA fibers on the capturing of *Escherichia coli* and *Staphylococcus aureus.*

materials prepared

For the introduction of the tertiary amino group onto the fiber, the conversion of DEA increased with the reaction time. The fibers with the DEA conversion from 24% to 93%, i.e., the density of the DEA group from 0.8 mmol/g-product to 2.7 mmol/g-product, were obtained. The final DEA group density for the beads was 2.5 mmol/g-product.

Comparison of the capturing rate of microbial cells among the DEA-EO and DEA-EA fibers, and DEA beads

Both the DEA-EO and DEA-EA fibers with DEA conversion of 30, 69 and 93% were brought into contact with *E.coli* in batch mode, and the results showed that though both fibers captured *E.coli*, density of the DEA group and the coexisting of EA group had no significant influence on the capturing of *E.coli* (Fig. 2a and 2b). Whereas, for the DEA-EO and DEA-EA fibers (DEA conversion: 30, 69 and 93%) tested against *S.aureus* in the same batch mode, the DEA-EO fiber with a higher conversion of DEA captured the Gram-positive bacteria faster (Fig. 2c). However, when the DEA-EO fiber was hydrophilized with EA (DEA-EA fiber), the mode for capturing of microbial cell was retarded (Fig. 2d).

It is usually recognized that microbial cells have predominantly negative charges, and proposed that microbial cells can be adsorbed on solid surfaces by electrostatic interaction. And, the Gram-positive microbial cells (*S.aureus*, 2.56×10^{-16} g·eq/cell) have a higher surface charge density than the Gram-negative microbial cells (*E.coli*, 0.24×10^{-16} g·eq/cell) (Kawabata, 1992). The graft

Table 1. Comparison of capturing rate constant between crosslinked-type and grafted-type materials for the capturing of *S. aureus* in batch mode.

		This study	
	Tashiro	DEA beads	**DEA(93)-EO fibers**
type of material	chloromethylated, divinylbenzene crosslinked polystyrene bead	ethylene glycol dimethacrylate crosslinked GMA bead	**fiber containing graft chain**
functional group	polyethyleneimine	diethylamine	**diethylamine**
polymer structure	crosslinked	crosslinked	**grafted**
surface area (m^2)	1.08	0.10	**5.54×10^{-3}**
functional group density (mmol/g-product)	1.00	2.89	**2.69**
bacteria solution condition	in sterilized physiological saline	in sterilized water	**in sterilized water**
temperature (°C)	37	37	**37**
capturing rate constant, k*[1] (m/s)	1.18×10^{-9}	3.83×10^{-9}	**1.16×10^{-6}**

*[1] $k=-(V/A)(1/t)\ln(C/C_0)$ where V, A, t, C and C_0 are the volume of viable cell suspension (m^3), the contact surface area (m^2), the contact time (s), the viable cell number (CFU/mL) at contact time t and the initial viable cell number (CFU/mL), respectively.

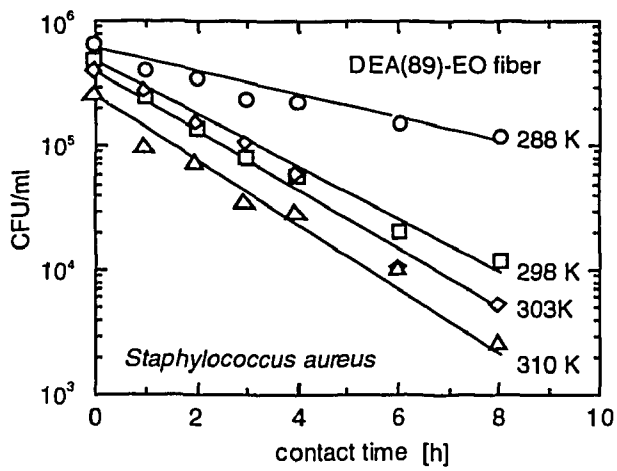


Fig 3. Temperature effect of DEA(89)-EO fiber on the capturing of *Staphylococcus aureus*.

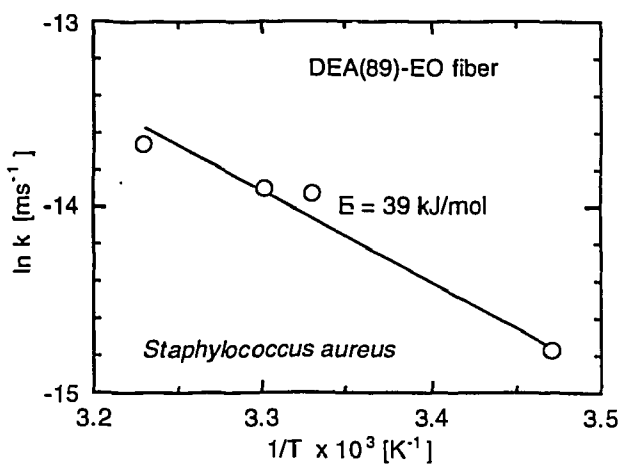


Fig 4. Arrhenius plot: microbial-cell-capturing rate constant of DEA(89)-EO fiber (ln k) *vs* 1/T.

chains of the DEA-EO fiber are partially protonated in water at pH 7 to yield cationic nitrogen atoms. Therefore, it may conclude that the microbial cells can be captured by the DEA-EO fiber mainly due to electrostatic interaction during the contact with the *S.aureus* cell suspension. As a result, the DEA-EO fiber prepared by RIGP exhibited good microbial cell capturing activity.

Similar experiment was also performed for the DEA beads. A slow capturing rate was observed for the DEA beads as compared to the DEA(93)-EO fiber. Comparison of capturing rate

constant among the polyethyleneimine beads of Tashiro (1991), the DEA beads and the DEA(93)-EO fibers is shown in Table 1. The DEA group densities for the DEA beads and DEA-EO fiber (2.6 and 2.7 mmol/g-product, respectively) used in the experiment were almost the same. Also, it was found that the DEA(93)-EO fiber has a capturing rate constant 1000-fold higher than the DEA beads (Table 1).

Temperature effect for capturing of S. aureus by DEA-EO fiber

Figure 3 shows that the microbial-cell-capturing rate of DEA(89)-EO fiber increased as the temperature is raised from 15 to 37 °C. The activation energy of the microbial-cell-capturing action for the DEA(89)-EO fiber was 39 kJ/mol (Fig. 4). This level of activation energy indicated that the interfacial interaction between *S. aureus* and the DEA(89)-EO fiber involved not only a physical process but a chemical reaction process as well. Kourai et al. (1985, 1986) reported that the activation energies of bactericidal action against *E. coli* for 12-alkylpyridinium iodides and 12,12'-dialkyl-γ,γ'-dipyridinium diiodides were 115 and 127 kJ/mol, respectively.

CONCLUSION

The coexisting of the epoxy group is an important factor for the capturing of *S. aureus* onto the interface containing cationic graft chains. The grafted-type DEA-EO fiber captures the cells 1000-fold faster than the crosslinked-type DEA beads. And, from the activation energy obtained, this study shows that the interaction between the DEA-EO fiber and *S. aureus* involved both the physical process and chemical reaction process.

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