Bacterial Adhesion on Rifampin-immobilized Polyurethane Surface

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INTRODUCTION

Bacterial adhesion to the surface of biomaterials is the crucial event in the initiation of a device infection. The adhesion mechanisms were not well known yet, but it has been generally recognized that bacterial adhesion to the polymeric surfaces may be dependent on degree of hydrophobicity of the bacterial surface[1], production of slime [2],plasma proteins such as fibronectin, fibrinogen[3], and platelets[4] , cells and tissues living on polymeric surfaces[5].

The device infection has been widely investigated toward prevention of bacterial adhesion on polymeric surfaces.

It has been tried to prevent of bacterial adhesion on biomaterials using incorporation of antibiotics to polymer [6] or use of electromagnetic filed with antibiotics[7].

In the previous study, we have reported on the rifampin-immobilized polyurethane that has an antibacterial effect against *S.aureus* in the planktonic condition[8].

A new approach analyizing the antibacterial effect of rifampin-immobilized polyurethane was developed to reveal the mechanism of bacterial adhesion on rifampin-immobilized polyurethane surface. We will demonstrate that the rifampin-immobilized polyurethane reduce bacterial adhesion in comparision to plain polyurethane in this paper.

MATERIALS AND METHODS

Preparation of bactria

In the experiments of bacterial adhesion to the surface of PU films, we used gram- positive Rifampin- sensitive strains of *S.aureus* (ATCC 27735) and *S.epidermidis* RP12(ATCC35983).

The organisms were respectively grown in nutrient broth(NB,Difco Laboratories.) and tryptic soy broth(TSB,Difco Laboratories.) for 3 hr [8].

Preparation of polyurethane film

Three kinds of polyurethane films were prepared ; PU(10 x 20 x 1 mm), PU incorporated with Rifampin(10 x 20 x 1 mm), Rifampin-immobilized PU(10 x 20 x 1 mm). PU and PU incorporated with Rifampin were used as control, and Rifampin-immobilized PU films were prepared by chemical bonding with HMDI(1,6-Diisocyanatohexane).

Bacterial adhesion tests

Log phase cultures of *S.aureus* and *S.epidermidis* were used in adhesion tests. The cells were diluted at the concentration of 0.5-3 x 10^6/ml with NB or TSB and added three samples into culture tubes containing the bacterial suspensions.

Adhesion tests were conducted for 1,3,24,48,72 hours at 120rpm,37`C in shaking incubator. After incubation , samples were washed twice in 0.01M PBS(pH7.4) to remove nonadherent organisms from the sample surface, and placed in culture tube containing 3ml of sterilized PBS. The bacterial cells adhered on the polymeric surfaces were detached from samples by vortex mixing for 2 min.

Also, to investigate that the effect of serum on the

bacterial adhesion, we incubated three samples in BCS(bovine calf serum) and PBS(phosphate buffered saline) for 3 hr.

Samples exposured to serum were transferred into sterilized PBS(3ml), and rinsed gently twice with PBS. All samples were exposured separately to bacterial suspensions(2.11 X10^5/ml for *S.aureus.*, 5 x 10^5/ml for *S.epidermidis.*) in a static condition at 37`C for 24hrs. After incubation, they were assayed according to the previous detachment method . The number of viable bacteria was determined by the plate count method.

Scanning Electron Microscopy

PU films were immersed in a fixative solution of 2.5% glutaraldehyde in PBS(0.01M, pH7.4)for 24hrs at 4`C, and then dehydrated through series of washings in increasing ethanol concentrations (50,75, and 100%) for 10 min in each concentration, and two times in 100% ethanol.

Samples were dried in critical point dryer(LADD Research Ind, Inc.,USA) at 44`C for 100 minutes.

Dried samples were coated with gold in a gold sputter (fine coat ion sputter JFC-1100,Jeol LTD, Japan) and examined by using a Jeol JSM-35CF scanning electron microscope(USA) at an accelerating voltage of about 25kV.

RESULTS AND DISCUSSION

Effect of incubation time on bacterial adhesion

Figure 1 shows the amount of *S.aureus* adhered to three kinds of PU films. The results show no significant difference when the bacteria was incubated with rifampin-incorporated PU film and rifampin-immobilized PU for 24 hr, but the number of bacteria adhered on rifampin-immobilized surface reduced to a half in comparison with plain PU.

The amount of adhered *S.epidermidis* to rifampin-immobilized PU also is reduced [Figure 2].

We examined the effect of adhesion time on bacterial adhesion to three kinds of PU films. In an initial step of incubation, the amount of bacterial adhered to all films has no significant difference.

However, it shows difference in the number of *S.aureus* after 24hrs of incubation [Figure 3].

Especially, the amount of *S.epidermidis* adhered on rifampin-immobilized PU surface reduced more significantly a little than those of PU and rifampin-incorporated PU surfaces and the effect of decrease in bacterial adhesion on rifampin-immobilized PU maintained until 72hrs [Table 1].

Effect of serum on bacterial adhesion

Figure 4 shows the reduction of amount of bacterial cells adhering PU films . The influence of serum in a bacterial adhesion was not showed on PU film, but rifampin-incorporated PU and rifampin-immobilized PU showed that the bacterial adhesion influences by serum in *S.epidermidis.*

However, the remarkable reduction of bacterial adhesion to PU by serum not revealed in *S.aureus.* Through the these results , we could suppose that the serum protein may not a crucial factor on bacterial adhesion to PU.

Observation of the surface of polyurethane after exposure to Staphylococci

The surfaces of three samples were examined by SEM before and after 24 hrs of incubation in broth cultures of Staphylococci. Figure 5 shows the surfaces of plain PU, rifampin-incorporated PU, and rifampin-immobilized PU before exposure to a bacterial suspension. Both polyurethane and rifampin-incorporated PU were relatively smooth and uniform, but the surface of rifampin-immobilized PU was very rough and irregular.

Figure 6 shows the appearance of the surface of PU films exposured to *S.epidermidis* for 24 hrs. *S.epidermidis* is not shown in both of rifampin-incorporated PU, and rifampin-immobilized PU except plain PU.

This experiment demonstrates that the rifampin-immobilized PU is effective in preventing adhesion of Staphylococci on the PU surface. This inhibitory effect was continued until 72 hrs and maintained under influence of serum protein.

In this experiment, the functional activity of rifampin-immobilized PU was evaluated by the bacterial adhesion ,but only other assays for anti-infective beheavior will be investigate in further study.

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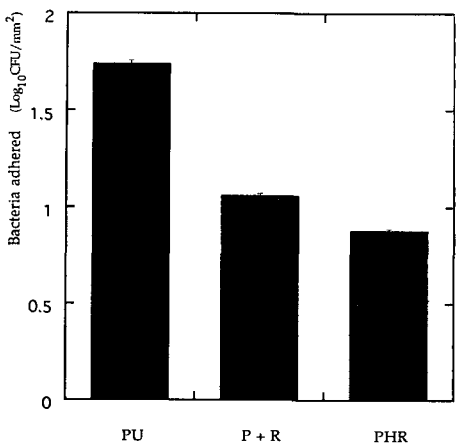


Fig 1. Adhesion of *S.aureus* to PU,rifampin-incorporated PU(P+R),and rifampin-immobilized PU(PHR)

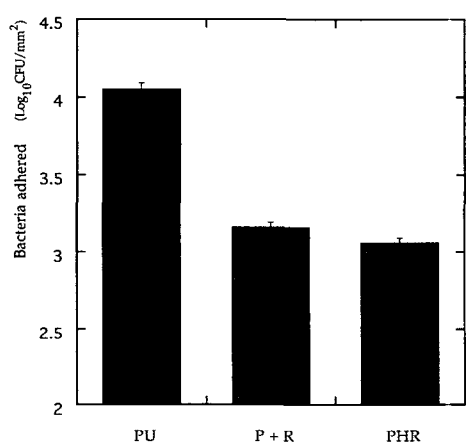


Fig 2. Adhesion of *S.epidermidis* to PU,rifampin-incorporated PU(P+R),and rifampin-immobilized PU(PHR)

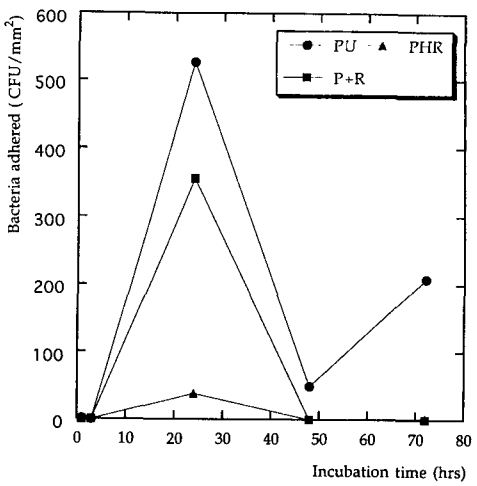


Fig 3. Effect of incubation time on bacterial adhesion; Adhesion of *S.aureus* to PU,rifampin-incorporated PU(P+R),and rifampin-immobilized PU(PHR)

Fig 5 . Scanning electron microscope micrographs of the surface of three different polymers before exposure to bacteria
A: PU
B: Rifampin-incorporated PU
C: Rifampin-immobilized PU

Table I. – Effect of incubation time on bacterial adhesion ; Adhesion of S.epidermidis to three kinds of polyurethanes

Incubation time (hrs)	No. of organism adhering(CFU/mm2) *		
	PU	Rifampin-incorporated PU	Rifampin-immobilized PU
1	5.27+/- 2.92	0.02+/-0.01	0.02+/-0.01
3	3.52+/-0.24	0.00+/-0.01	0.00+/-0.01
24	22195.43+/-2043.26	204.33+/-94.70	2.12+/-0.07
48	895.67+/-20.89	49.67+/-2.49	21.00+/-3.56
72	1793.48+/-0.09	2.14+/-0.29	1.12+/-0.23

* Number of organism presented as means+/-SD of 3 experiments, each with 3 polymers

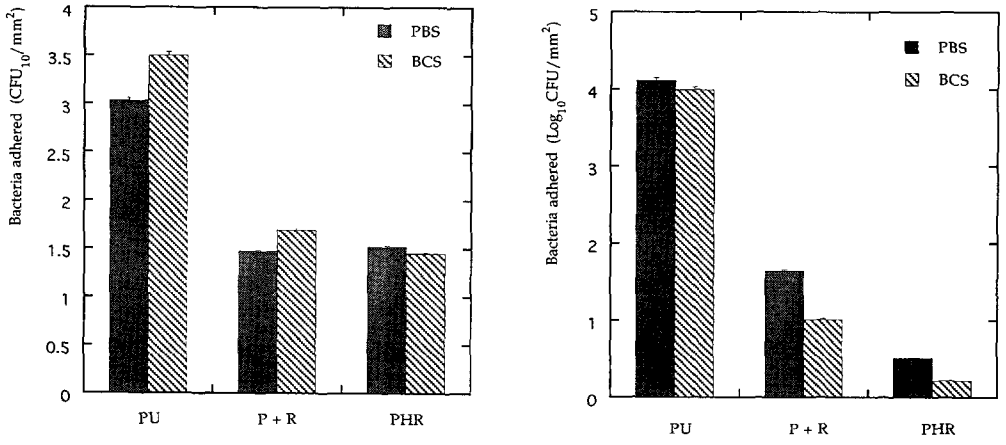
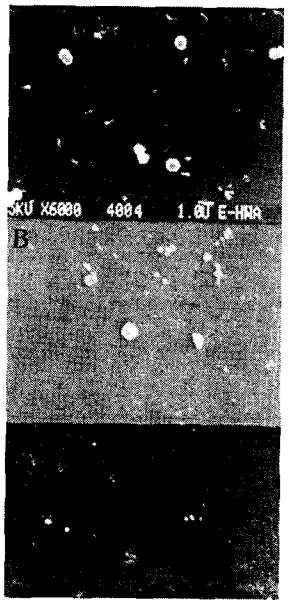


Fig 4. Effect of serum on bacterial adhesion; Adhesion of *S.aureus*(left) and *S.epidermidis*(right) to PU,rifampin-incorporated PU(P+R),and rifampin-immobilized PU(PHR)

Fig 6. Scanning electron microscope micrographs of the surface of three different polymers after 24hr of exposure to *S.epidermidis*
A: PU
B: Rifampin-incorporated PU
C: Rifampin-immobilized PU



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