표면이 개질된 폴리우레탄의 세균억제효과

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'Microbial Inhibition Effects by Surface Modified Polyurethane

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INTRODUCTION

Staphylococcus aureus, *Staphylococcus epidermidis*, and *Pseudomonas aeruginosa*. have been recognized as a pathogens which involved in infection associated with prosthetic implants and medical devices [1] and it may give rise to serious problems such as severe morbidity, amputation, or death [1] since these infections tend to resistant to treatment and persist until the implant is removed [2].

Recently, Gershon et al. have reported the prevention of bacterial colonization on polyurethane in vitro by incorporated antibacterial agent [3] and we also tried to find a new antimicrobial treatment method on polyurethane surface.

We mainly focused on the biomaterial-centered infection by *S. aureus*.(a major cause of implant infections) and found that Rifampin immobilized by carbamate linkage can be a candidate for the antimicrobial treatment on the surface of polyurethane.

MATERIALS AND METHODS

Bacterial strains and growth conditions

Staphylococcus aureus ATCC27735 Taniuchi strain Foggie. was used and the bacteria was cultured in NB(Nutrient Broth, Difco.) at 37°C for 18 hr. to obtain log phase culture.

Escherichia. coli. and *S. pneumoniae*. were used as the control. *E. coli*. was grown in the same medium as *S. aureus*. and *S. pneumoniae*. was anaerobically cultured in Todd Hewitt Broth(Difco.).

Preparation of modified PU discs

PU sheets were prepared by solvent-casting method and punched in discs shape with 10 or 12 mm diameter for disc diffusion test.

Two kinds of synthetic oligodeoxythymidine (oligodT 5 mer, 10 mer, Korea Biotech Inc.) were used as a linker between PU surface and antibiotics (Rifampin) from the possibility that the exoenzyme nuclease S1 from the *S. aureus*. can cleave the linker so as to release the attached antibiotic drug. OligodT was covalently immobilized on the PU surface by chemical treatment with HMDI (Hexamethylene-diisocynate). Rifampin was linked to another terminus of oligodT with succinic acid or HMDI. PU discs without surface treatment were used for negative control. All sample discs except for PU control were washed in methylene chloride for 2 days at room temperature, and washed in 100% ethanol for 1 day in the dark condition. Prior to the disc diffusion test, all discs were sterilized by ethylene oxide gas.

In order to evaluate the effect of sterilization method, we sterilized the rifampin immobilized PU directly with HMDI by EO gas, by 75% ethanol for 1 min and by UV irradiation for 30 min for each side of disc. And tested the antibiotic effect of immobilized rifampin

Antibacterial studies

The Disc diffusion method was used for the investigation of microbial growth inhibition effects of the modified PU. Broth cultured *S. aureus*. and *E. coli*. were inoculated on nutrient agar plate and *S. pneumoniae* were inoculated on blood agar plate and

smeared thoroughly on the surface of culture plates. Within 15 minutes after inoculation, the modified PU and PU control discs were gently mounted on the plate inoculated with bacterial suspension using a sterilized forceps. The nutrient agar plates with sample discs were incubated aerobically and the blood agar plates were incubated anaerobically at 37°C for overnight. We measured the diameters of bacterial inhibitory zone and compared its size among the modified discs and controls.

RESULTS AND DISCUSSION

In this study, we found that all of the modified polyurethane has an inhibitory activity against bacteria but has no expected specificity. These antibacterial effects are shown in [Table 1].

[Table 1]. Newly modified PU and its microbial growth inhibitory activity

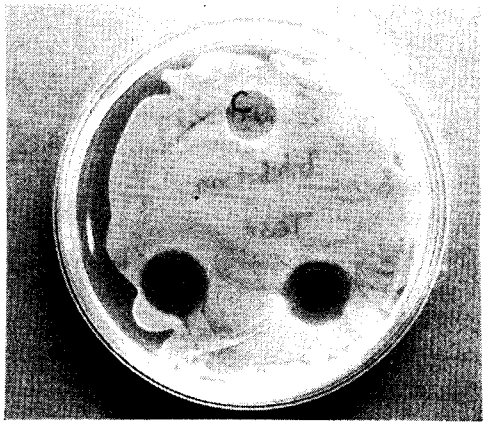
Disc/Bacteria	*S. aureus*	*E. coli*	*S.pneumoniae*
PU control	-	-	-
PU-HD-Rif	+	-	+
PU-HD-dT(5)-SA-Rif	+	-	+
PU-HD-dT(10)-SA-Rif	+	-	+
PU-HD-dT(5)-SA-Rif	+	-	+
PU-HD-dT(10)-SA-Rif	+	-	+

* PU: polyurethane, HD: hexamethylenediisocyanate, SA: succinic acid, Rif.: rifampin, (5): oligodT 5 mer, (10): oligodT 10 mer, +: clear zone formed, -: no clear zone

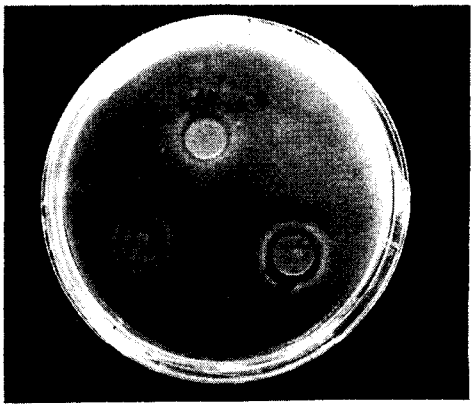
Control and all the rifampin immobilized PU discs showed no antibiotic effect against *E. coli*. which is insensitive to rifampin. The observed insensitivity means that no other antibiotic or bactericidal chemical substances other than rifampin were on the modified discs.

All the rifampin immobilized PU discs showed antibiotic effect against *S. aureus*. [Figure 1&2] and *S. pneumoniae*.[Table 1].

We expected that only the rifampin immobilized disc with ester linkage shows the inhibitory effect. However, the results showed that antibiotic effect against bacteria can be exerted by both the rifampin directly immobilized with HMDI and that immobilized



[Figure 1] The Antibiotic Effect of PU-HD-dT-SA-Rif on *S. aureus*, C:control, 5:PU-HD-dT(5)-SA-Rif, 10:PU-HD-dT(10)-SA-Rif



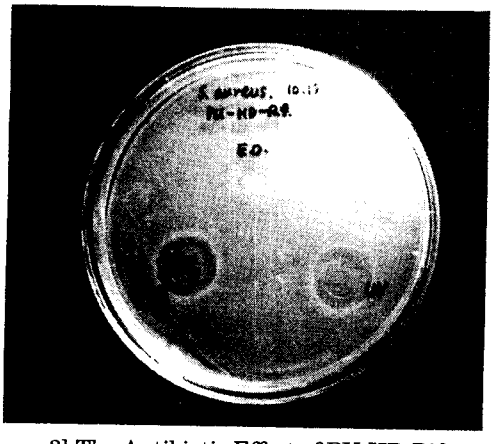
[Figure 2] The Antibiotic Effect of PU-HD-dT-HD-Rif on *S. aureus*, 5-SA:PU-HD-dT(5)-SA-Rif, 10-HMDI:PU-HD-dT(10)-HD-Rif, 5-HMDI:PU-HD-dT(5)-HD-Rif

with HMDI-oligodT-HMDI. From the above results, we found that both the ester linkage between succinic acid and rifampin, and the carbamate linkage between HMDI and rifampin can be cleaved by the enzymes of *S. aureus*. and *S. pneumoniae* or can be hydrolyzed in the culture medium.

The minimum 90% inhibitory concentration of rifampin against *S. aureus*. is so low as less than 0.03μg/ml. Extremely small amount of residual rifampin adsorbed on the modified PU discs can cause bias. To rule out the possibility of the effect of residual rifampin, we additionally washed the PU-HMDI-Rifampin discs in methylene chloride for 2 days and successively in ethanol(75%) for 1 day. After dried *in vacuo* for 2 days, the sample discs were tested again. .They also showed antibiotic effect.

The effect of sterilization method on the antibi-

otic activity of rifampin can be mildly changed by EO gas[Figure 3 & Table 2.].



[Figure 3] The Antibiotic Effect of PU-HD-Rif
EO:ethylene oxide gas sterilization, EtOH:75% ethanol sterilization for 1 min, UV:UV sterilization for 30 min each side

[Table 2] The Effect of Sterilization Method on the Antibiotic Activity of Rifampin

Sterilization Method	Size of Inhibitory Zone
Ethylene Oxide	3.5 mm
75% Ethanol	5.0 mm
UV Irradiation	5.0 mm

The diminished antibiotic activity of rifampin in case of EO sterilization is thought to be due to high temperature during the process of EO sterilization.

We think that the labile carbamate linkage resulted from the reaction of isocyanate and rifampin can be a candidate for the covalent linkage for the immobilization of rifampin on the polymer surface if the stability of linkage is more improved. Further study is necessary to evaluate the mechanism and extent of instability of carbamate linkage between isocyanate and Rifampin.

REFERENCES

[1].Dankert.J., Hogt A. H. and Feijen, J. biomedical polymers:bacterial adhesion, colonization and infection, CRC Crit. Rev. Biocompat., 2, 219-301(1986).

[2]. Dougherty S. H. and Simmons R. L., "Infections in prosthetic devices-part I ", Curr. Prol. Surg., 19, 221-264(1982).

[3]. Gershon Golomb and Arie Shipgelman, Prevention of bacterial colonization on polyurethane in vitro by incorporated antibacterial agent, J. of Biomedical Materials Research, Vol. 25, 937-952(1991).