Inhibition of bacterial adhesion by tobramycin-impregnated PMMA bone cement

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We investigated the effect of tobramycin-impregnated polymethylmethacrylate (PMMA) bone cement on the adhesion and colonization of *Staphylococcus epidermidis*. The pattern of colonization was quantitated using plate count techniques and electron microscopy.

Colonization of the tobramycin-impregnated disc surface by adhesive bacteria was demonstrated but it was less than in the control disc. This finding suggests that tobramycin may reduce bacterial adherence and proliferation on the PMMA surface.

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Biomaterial-associated infections are believed to have some relation with preferential adhesive bacterial colonization. Recent studies have clearly implicated adhesive, slime-producing bacteria as causal agents in biomaterial or foreign body infections (Gristina and Costerton 1985). Previous studies showed that *Staphylococcus E-46* cells easily adhered to PMMA and produced a protective adherent biofilm (Oga et al. 1988). We now report the effect of tobramycin impregnated in PMMA on bacterial adherence and colonization.

Material and methods

Preparation of tobramycin-bone cement discs

Sterile tobramycin sulfate powder and sterile silicon bead mold which has 16 holes, 10 mm diameter, 3 mm depth (DOSAKA. E.M.CO.) were used. Tobramycin powder and PMMA bone cement were mixed in the proportion of 1.2 g tobramycin as the sulfate salt with one 40 g package of PMMA bone cement (Goodell 1986). The PMMA-tobramycin mixture was put into the mold with a 10 mL sterile syringe. After the cement became hard, discs were removed from the mold. Each disc was then put into envelopes and sterilized by ethylene oxide. Each disc weighed an average of 0.29 g and contained 5.9 mg tobramycin activity. For the dissolution study, three discs were put in a tube containing 10 mL PBS and incubated for 24 h at 37 °C.

The dissolution medium was collected and assayed at the end of each 24-hour period. Then the tube was washed thoroughly with 5 mL PBS. The rinsing solution was discarded and 10 mL PBS was added for the next 24-hour period. The tobramycin content of PBS was determined by Emit Tobramycin assay (Leung et al. 1979, Oellerich 1980). The tobramycin concentration in PBS dissolution from a disc was 73.2 ± 4.2 μg/mL initially, and 20.3 ± 2.8 μg/mL on Day 2.

Test system

The laminar flow colonization chamber used was a modification of the Robbins Device (MRD) developed by McCoy (1981). The MRD was connected with sterile rubber tubing to a reservoir (37 °C) containing 2.0 L of the liquid bacterial culture. The bacterial suspension was delivered from this reservoir at 60mL/h by a peristaltic pump.

Bacterial culture

A strain of *S. epidermidis* (SE-46), isolated by Yamamoto and Iwata (1986) from a biomaterial-centered infection, was used. The bacteria were cultured for 48h in 500 mL of Trypticase soy broth (TSB) without dextrose (Difco Laboratories, Detroit, MI, U.S.A.), supplemented with 0.5 percent gluconic acid (Sigma Scientific, St. Louis, MO, U.S.A.). 20 mL of bacterial suspension standardized by our method (Oga et al. 1988) was used to inoculate the 2.0 L batch culture that supplied the MRD, so that the reservoir delivered logarithmic cells to the MRD throughout the 24 h colonization period. The number of bacteria in the incubation suspension at the beginning of the experiment

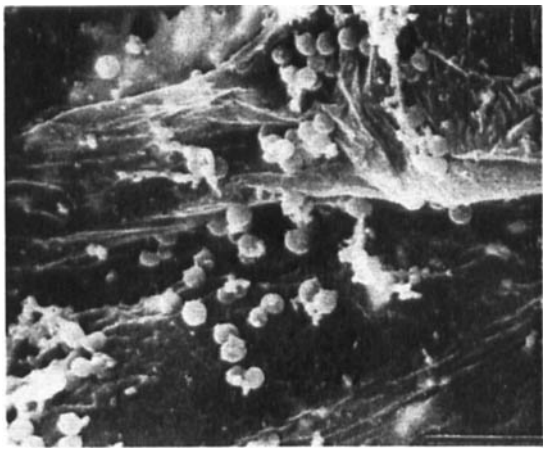

Figure 1. Scanning electron micrograph showing amorphous biofilm covering the PMMA without tobramycin. *Staphylococcus E-46* cells are observed within or on the biofilm. Bar, 5 microns.

Figure 2. Scanning electron micrograph of tobramycin-impregnated PMMA. Bacterial microcolonies are seen on the biofilm or within it. Bar, 5 microns.

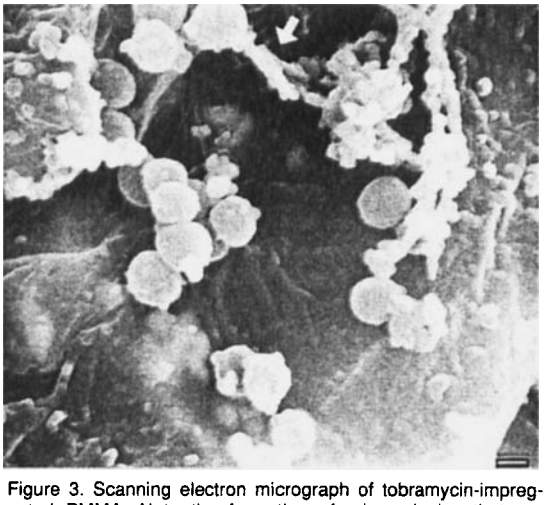

Figure 3. Scanning electron micrograph of tobramycin-impregnated PMMA. Note the formation of microcolonies that are anchored to the substratum by fibrous strands (arrow). Bar, 0.5 microns.

was about 6×10^7 to 10^8 cells/mL. The minimum inhibitory concentration (MIC) of tobramycin for cells taken from the seeding cultures was found to be 0.78 μg/mL.

Design of experiment

Experiment 1. 25 tobramycin-impregnated PMMA discs were placed in MRD. *Staphylococcus E-46* specimens cultivated in the reservoir were passed through the MRD for 24 h at a regular flow speed (60 mL/h). Specimen discs were collected 24 h after the reservoir was connected. 5 discs were examined by scanning electron microscopy (SEM). Each of the remaining 20 discs was immediately placed in 10 mL of PBS with pH 7.2 and rinsed five times in 10 mL of PBS. The number of viable bacteria adhering to each disc was determined by the colony counting method (Oga et al. 1988). This assay was performed five times.

The same experiment was done for the PMMA discs without tobramycin as a control.

Experiment 2. After 24 h incubation of discs in bacterial culture as in experiment 1, a PBS reservoir was connected to MRD and discs were rinsed for 24 h at a regular flow speed (60 mL/h). The number of viable bacteria adhering to each disc and the floating bacteria in PBS were determined in the same way as in experiment 1.

Scanning electron microscopy

The discs were immediately placed in 0.1 M cacodylate buffer containing 5 percent glutaraldehyde and 0.15 percent ruthenium red and incubated at room temperature for 2 h. Then samples were developed by the method described before (Gristina et al. 1985, Oga et al. 1988) and examined with a Hitachi S 700 scanning electron microscope.

Results

Observation of SEM showed that the surface of the PMMA without tobramycin was heavily colonized by *Staphylococcus E-46* cells and covered by a thick adherent biofilm (Figure 1). PMMA-containing tobramycin surface was also colonized by these bacteria, which were observed within or on the biofilm (Figures 2 and 3).

Experiment 1. The number of bacteria adhering to the PMMA-containing tobramycin in the five different assays varied from $(5.1 \pm 0.7) \times 10^3$ to $(10.5 \pm 1.2) \times$

Table 1. Experiment 1. Adhesion of *S. epidermidis* to PMMA exposed to a culture medium for 24 h

Assay	Amounts of adhering cells[a]		Adherent ratio
	Tobramycin-impregnated (ten thousands)	Controls (thousands)	
1	15.7 *2.6*	10.5 *1.2*	14
2	10.6 *0.9*	5.1 *0.7*	21
3	11.1 *1.1*	5.8 *1.0*	19
4	11.1 *1.6*	6.9 *1.1*	16
5	13.3 *2.1*	7.1 *1.0*	19

[a]Mean *SE* of results in 20 PMMA discs

Table 2. Experiment 2. Adhesion of *S. epid.* to PMMA exposed to a culture medium for 24 h and rinsed with PBS for 24 h

Assay	Amounts of adhering cells[a]		Adherent ratio
	Tobramycin-impregnated (thousands)	Controls (hundreds)	
1	10.1 *1.1*	2.3 *0.6*	44
2	13.5 *1.1*	3.3 *0.8*	41
3	16.4 *1.1*	2.1 *0.6*	78
4	15.5 *1.6*	1.6 *0.5*	97
5	18.4 *1.8*	2.3 *0.6*	80

[a]Mean *SE* of results in 20 PMMA discs

10^3 cells and to the PMMA without tobramycin was $(11.1 \pm 1.1) \times 10^4$ to $(15.7 \pm 2.6) \times 10^4$ cells. The adherent ratio was defined as the number of bacteria on PMMA without tobramycin divided by the number of bacteria found on PMMA impregnated with tobramycin under the same experimental conditions. This result shows that 14 to 21 times more bacteria adhere to PMMA than to tobramycin-impregnated PMMA (Table 1).

Experiment 2. The number of bacteria adhering to the PMMA-containing tobramycin was $(1.6 \pm 0.5) \times 10^2$ to $(3.3 \pm 0.8) \times 10^2$ cells and to the PMMA without tobramycin was $(10.1 \pm 1.1) \times 10^3$ to $(18.4 \pm 1.8) \times 10^3$ cells. The adherent ratio shows that 41 to 97 times more bacteria adhere to PMMA than to tobramycin-impregnated PMMA (Table 2).

Discussion

Recent studies have indicated that biomaterials act as substrata for adhesive bacterial colonization; initial microbial surface contact is followed by progressive adhesion and aggregation (Gristina et al. 1985). Exopolysaccharide polymers, which are critical for microbial aggregation and, in part, for surface adhesion, may sequester nutrients and enhance resistance to host defense mechanisms and antibiotics (Baltimore and Mitchell 1980, Nickel et al. 1985, Naylor et al. 1990). Our earlier experiment showed that surgical grade PMMA was colonized to a greater extent than either HDP, SUS 316L, $AL_2\ O_3$ and colonized bacteria covered by exopolysaccharide were observed by scanning electron microscopy (Oga et al. 1988). The result suggested that reduction of bacterial adherence to PMMA would be one way to prevent infection of an artificial joint. Good results were reported with antibiotic-impregnated bone cement for revision of infected endoprostheses (Bucholz et al. 1981, Bucholz et al. 1984). The mixing of various antibiotics with PMMA bone cement has been investigated, but gentamicin-impregnated bone cement is the most popular. The mechanism for the release of antimicrobials from PMMA bone cement is controversial. Some investigators have suggested that the drug diffuses through the matrix of the cement (Elson et al. 1977, Bayston and Milner 1982), others that it is released from the surface through holes and pores in the cement (Marks et al. 1976, Baker and Greenham 1988). Hughes et al. (1979) tested cephalosporins mixed with CMW Type 1 bone cement in vitro and showed that they could prevent the growth of bacteria near the surface of the impregnated cement and they remained active for several weeks. Marks et al. (1976) and Elson et al. (1977) noted that gentamicin leached from Palacos acrylic cement in a better way than other antibiotics did. Hoff et al. (1981) studied the elution of penicillin and gentamicin from PMMA. The results of this study suggest that the elution of penicillin from Palacos is superior to that of gentamicin. Goodell studied the release of tobramycin from PMMA and concluded that the release of tobramycin followed a predictable pattern and that variation between the individual samples was small. We prepared tobramycin-impregnated bone cement by the Goodell method (Goodell et al. 1986) and investigated its effect on bacterial adherence and colonization of bone cement in vitro.

Examination by SEM showed that adherent bacterial cells and exopolysaccharide biofilm were observed on the surface of tobramycin-impregnated PMMA. The number of adherent and colonizing bacteria to tobramycin-impregnated PMMA was a little less than the controls in experiment 1. This indicated that tobramycin-impregnated in PMMA could not significantly prevent bacterial adhesion and colonization.

However, in experiment 2, adherent bacteria continued to decrease until they were about 40–100 times less than the controls. We assume that the high concentration of tobramycin released from PMMA can block the bacterial adherence and colonization and kill some of the adherent bacteria.

The current results indicated that tobramycin-impregnated PMMA bone cement could not completely eradicate the adherent bacteria covered by exopolysaccharide biofilm. However, it was effective in reducing bacterial adherence and colonization on PMMA. Thus tobramycin-impregnated PMMA bone cement would be useful in preventing infection after total joint replacement.

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