Biofilm Formation on Silicone Tympanostomy Tubes With Polyvinylpyrrolidone Coating

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Objective: To determine whether biofilm formation on silicone tympanostomy tubes (TTs) is prevented by polyvinylpyrrolidone (PVP) coating.

Design: In vitro microbiologic study.

Subjects: Silicone TTs with and without a PVP coating.

Intervention: The TTs were exposed to blood or phosphate-buffered saline and cultured with *Pseudomonas aeruginosa* or *Staphylococcus aureus*. After 4 days, antibiotics were added to kill planktonic bacteria. Biofilm formation was assessed by quantitative bacterial counts and scanning electron microscopy.

Results: Human blood enhanced *S. aureus* biofilm formation on TTs with and without PVP (P < .001). *Staphylococcus aureus* biofilm formation was similar on TTs with and without PVP coating. *Pseudomonas aeruginosa* biofilm formation was less on TTs with PVP coating after exposure to phosphate-buffered saline (P = .04), but this difference was not significant after blood exposure (P = .19).

Conclusions: Polyvinylpyrrolidone coating of TTs imparts resistance to *P. aeruginosa* biofilm formation. The clinical impact of PVP on TTs may be attenuated by exposure to blood, but this will require study in clinical trials.


Biofilm formation on tympanostomy tubes (TTs) may lead to refractory otorrhea and TT occlusion.1-3 The TT composition and surface coatings have been shown to affect TT occlusion and microbial adherence.4-7 Polyvinylpyrrolidone (PVP) hydrogel coatings have been shown to reduce bacterial adherence, the first step in biofilm formation, by more than 90%.8 Silicone TTs coated with PVP are commercially available (Microgel, Medtronic ENT, Jacksonville, Florida). The aim of this study was to determine whether commercially available PVP-coated TTs could reduce biofilm development by pathogens commonly found in posttympanostomy otorrhea, *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

**STUDY METHODS**

**STUDY DESIGN**

Silicone TTs, with and without PVP coating, were exposed to phosphate-buffered saline (PBS) or blood because blood has been shown to promote biofilm formation on TTs.2 The blood was allowed to dry overnight. The TTs were cultured with *P. aeruginosa* or *S. aureus*, common pathogens in post-TT otorrhea,10 in microtiter plates for 4 days; antibiotics were then added for 24 hours to kill planktonic bacteria. Biofilm formation was assessed by quantitative bacterial counts and scanning electron microscopy. There was a total of 8 test groups, with 25 TTs per group (22 TTs for quantitative bacterial counts and 3 TTs for microscopic analysis). Because of the large numbers of samples involved, the study was run in 3 batches, with equal distribution of samples. Each batch included equal numbers of each of the test organisms, TTs with and without PVP, and PBS or blood exposure (Table 1). Study variables (eg, culture duration and sonication time) were optimized through earlier pilot trials. No humans or animals were used in this study.

**TTs AND PREPARATION**

Sheehy-type collar-button TTs (Medtronic ENT) were used. Silicone TTs with PVP coating (Microgel, product number 1083303) were compared with plain silicone TTs (product number 1028146). Half of each TT type was immersed in human blood and half was immersed in PBS for 24 hours. The TTs were removed and allowed to dry overnight. A total of 100 of each TT type was used, divided evenly among the fluid and bacterial exposure groups (ie, 25 per group; Table 1).

**BACTERIAL STRAINS AND PREPARATION**

*Pseudomonas aeruginosa* (strain PAO1) and *S. aureus* (ATCC strain 29213) were used because...
these strains have been studied extensively and are known bio-
film formers. Culture media were tryptic soy broth (MP Bio-
medicals, Solon, Ohio) and tryptic soy agar (Becton Dickin-
son, Sparks, Maryland). Cultures were grown in 96-well
microtiter plates maintained at 37°C for 4 days because pilot
studies had revealed mature biofilms with both S aureus and P
aeruginosa after 2 days. Gentamicin sulfate, 20 µg/mL (Sigma,
St Louis, Missouri), or oxacillin sodium, 1 mg/mL (Fluka, Stein-
heim, Germany), was added to the culture media to eradicate
plankiotic P aeruginosa and S aureus, respectively.

BIOFILM ANALYSIS

Following antibiotic treatment, TTs were washed 4 times for
10 minutes by adding 150 µL of PBS to each well. Phosphate-
buffered saline washes were aspirated using a sterile glass Pas-
teur pipette (Fisher Scientific, Fair Lawn, New Jersey). The TTs
were then transferred to 15-µL-flip-top conical tubes (Thermo
Scientific, Rochester, New York) containing 5 mL of PBS
with 5 ppm of Tween-80 (Fisher Chemical, Fair Lawn, New
Jersey). The conical tubes were placed into a water bath and
sonicated for 5 minutes, with serial 1-minute sonication expo-
ures separated by a 1-minute rest. After sonication, the tubes
were vortexed at the highest setting (setting 8) for 15 seconds,
serially diluted, and spread plated onto tryptic soy agar in tri-
plicate. Plates were incubated for 18 to 24 hours at 37°C, and
colonies were counted.

SCANNING ELECTRON MICROSCOPY

Representative TT samples not processed for bacterial counts
were fixed in 2 mL of Trumps fixative (1% glutaraldehyde and 4% for-
maldehyde in PBS) and stored at 4°C until processed. Speci-
mens were washed 3 times with PBS for 10 minutes and then
fixed for 1 hour in 1% osmium tetroxide in PBS (Electron Mi-
croscopy Sciences, Hatfield, Pennsylvania). Specimens were
washed once with PBS and 3 times with deionized water for 10
minutes each. Specimens were dehydrated in ethanol series for
10 minutes each (25%, 50%, 75%, 95%, and 100%) and then hexa-
methyldisilazane (Electron Microscopy Sciences) for 5 min-
utes. Specimens were allowed to air dry overnight. Specimens
were sputter-coated with gold/palladium with argon gas (Desk
II sputter coater; Denton Vacuum USA, Moorstown, New Jer-
sy) for 45 seconds and stored under vacuum until imaged.
Imaging was completed at 10 kV at a working distance of 15 cm.

STATISTICAL ANALYSIS

Sample means calculated to be greater than 2 SDs from the total
mean for each trial per TT type were considered outliers and
removed from the overall statistical analysis (Table 2). Data
were analyzed using t test (1-tailed) and 1-way and 2-way analy-
sis of variance followed by t test for comparisons of means. A
statistical value of P ≤ .05 was considered significant. All sta-
tistical analyses were carried out using JMP 7.0 (SAS Institute,

RESULTS

Bacterial counts were uniform in each test condition, with
few being considered outliers and excluded from analy-
sis (Table 2). There was no net effect from the 3 trials.
There were differences in the quantitative counts be-
tween trials; however, the trends observed in 1 trial mir-
rored those in the other trials.

After 4 days in culture, P aeruginosa formed mature
biofilms on both PVP-coated and uncoated silicone TTs
(Figure 1 and Figure 2). The TT coating had a sig-
nificant effect (P = .04) on biofilm development, favor-
ing the PVP coating. This effect was more pronounced
after exposure to PBS (P = .04) than blood (P = .19). There
was no overall difference between PBS and blood expo-
ure (P = .59).

Staphylococcus aureus also formed mature biofilms
af-
ter 4 days in culture (Figure 3 and Figure 4). The PVP
coating did not demonstrate a significant effect after ex-
posure to either blood (P = .20) or PBS (P = .26). Expos-
ure to blood did, however, strongly promote the devel-
oment of S aureus biofilms (P < .001).

COMMENT

Tymanostomy tubes may serve as a nidus for bacterial
adhesion and infection upon placement into the tym-
panic membrane. The topic of bacterial adherence to TT
materials has been the focus of study for more than 30
years. In vitro studies have demonstrated that more in-
ert TT materials and smoother surface preparations can
inhibit the adsorption of key bacterial binding proteins,
such as fibronectin, and the development of P aerugi-

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ficiently to allow bacterial adherence and aggregation. One might expect this to be more pronounced with dry blood; however, both dry and wet blood have been shown to promote *P. aeruginosa* biofilm development on TTs. The TTs placed by experienced otolaryngologists and treated with prophylactic ototopical preparations generally are not covered in blood to the degree that they were in this experiment. The clinical significance of the blood effect will be known only through clinical trials.

A number of factors should be considered when interpreting the effect of PVP coating on *S. aureus* biofilm formation. First, our bacterial challenge may have overwhelmed the PVP substrate. The PVP hydrogel dressings have been shown to be more effective at inhibiting concentrations of *S. aureus* on the order of logarithm 3.5 but not logarithm 7. Second, the effect of PVP may not be as pronounced or durable on *S. aureus*. The PVP dressings have been reported to have a lasting effect on *P. aeruginosa* biofilm and a transient effect on *S. aureus* biofilm. For long-term implants, such as TTs, only the durable effect is clinically relevant. Finally, hydrogel coatings have been reported to be nonuniform on the internal surfaces of catheters. Our imaging efforts focused on the external flange of the TTs and did not assess the uniformity of the PVP hydrogel coating. Imaging targeted at the internal barrel of the TT would be necessary to assess this issue.

Clinical trials are ultimately needed to demonstrate the clinical efficacy of new TT surface preparations. In vitro results with albumin- and phosphorylcholine-coated TTs looked encouraging but were not borne out in vivo. The effect of reducing surface imperfections and increasing surface antimicrobial activity may be reduced if middle ear mucus, blood, or cellular debris fouls the TT surfaces because adherent debris may serve as microbial binding sites. Avoiding biofilm development in vivo may depend on minimizing biofouling. Exposing TTs to blood before bacterial contamination pro-

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**Figure 1.** Scanning electron micrographs of silicone (A and B) and polyvinylpyrrolidone-coated silicone (C and D) tympanostomy tubes after exposure to human blood (A and C) or phosphate-buffered saline (B and D) and culture with *Pseudomonas aeruginosa*.

**Figure 2.** *Pseudomonas aeruginosa* colony counts on silicone and polyvinylpyrrolidone (PVP)-coated silicone tympanostomy tubes.
vided a relatively simplistic model of the biofouling that may be seen in vivo. Further insights may be gained by measuring adherence of plasma and mucus proteins to the TTs and testing the rate of TT occlusion. Coated TTs have demonstrated lower rates of occlusion, and PVP-coated TTs have shown the lowest rates of occlusion among coated or uncoated TTs. Thus, clinical trials with PVP-coated TTs seem warranted.

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Figure 3. Scanning electron micrographs of silicone (A and B) and polyvinylpyrrolidone-coated silicone (C and D) tympanostomy tubes after exposure to human blood (A and C) or phosphate-buffered saline (B and D) and culture with Staphylococcus aureus.

Figure 4. Staphylococcus aureus colony counts on silicone and polyvinylpyrrolidone (PVP)-coated silicone tympanostomy tubes.
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REFERENCES