Biofilm Formation on Silicone Tympanostomy Tubes With Polyvinylpyrrolidone Coating

Patrick J. Antonelli, MD; Edith M. Sampson, MS; Carolyn Ojano-Dirain, PhD

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. The TT composition and surface coatings have been shown to affect TT occlusion and microbial adherence. Polyvinylpyrrolidone (PVP) hydrogel coatings have been shown to reduce bacterial adherence, the first step in biofilm formation, by more than 90%. 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.

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. The blood was allowed to dry overnight. The TTs were cultured with P aeruginosa or S aureus, common pathogens in post-TT otorrhea, 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 number 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.

T Ts 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 then 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 PA01) and S aureus (ATCC strain 29213) were used because...
these strains have been studied extensively and are known biofilm formers. Culture media were tryptic soy broth (MP Biomedicals, Solon, Ohio) and tryptic soy agar (Becton Dickinson, 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, Steinheim, Germany), was added to the culture media to eradicate planktonic *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 Pasteur pipette (Fisher Scientific, Fair Lawn, New Jersey). The TTs were then transferred to 15-ml-clip-top conical tubes (Thermo Fisher 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 exposures 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 triplicate. 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% formaldehyde in PBS) and stored at 4°C until processed. Specimens were washed 3 times with PBS for 10 minutes and then fixed for 1 hour in 1% osmium tetroxide in PBS (Electron Microscopy 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 hexamethyldisilazane (Electron Microscopy Sciences, Hatfield, Pennsylvania). Specimens were then transferred to 15-mL flip-top conical tubes (Thermo Fisher Scientific, Fair Lawn, New Jersey). The conical tubes were placed into a water bath and sonicated for 5 minutes, with serial 1-minute sonication exposures 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 triplicate. Plates were incubated for 18 to 24 hours at 37°C, and colonies were counted.

**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 analysis of variance followed by t test for comparisons of means. A statistical value of *P* ≤ .05 was considered significant. All statistical analyses were carried out using JMP 7.0 (SAS Institute, Inc, Cary, North Carolina).

**RESULTS**

Bacterial counts were uniform in each test condition, with few being considered outliers and excluded from analysis (Table 2). There was no net effect from the 3 trials. There were differences in the quantitative counts between trials; however, the trends observed in 1 trial mirrored 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 significant effect (*P* = .04) on biofilm development, favoring 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 exposure (*P* = .59).

*Staphylococcus aureus* also formed mature biofilms after 4 days in culture (Figure 3 and Figure 4). The PVP coating did not demonstrate a significant effect after exposure to either blood (*P* = .20) or PBS (*P* = .26). Exposure to blood did, however, strongly promote the development of *S. aureus* biofilms (*P* < .001).

**COMMENT**

Tympanostomy tubes may serve as a nidus for bacterial adhesion and infection upon placement into the tympanic 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 inert TT materials and smoother surface preparations can inhibit the adsorption of key bacterial binding proteins, such as fibronectin, and the development of *P. aeruginosa* and *S. aureus* biofilms.

Because PVP coating has demonstrated inhibition of bacterial adherence on silicone and lower rates of infections associated with urologic implants, we sought to test the potential for this surface preparation to reduce biofilm development on TTs. Our observations suggest that the PVP coating does reduce biofilm formation by some pathogens under certain conditions. Exposing the TTs to blood may have fouled the PVP coating suf-
sufficiently 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 7.14 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.15 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.16 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 encouraging6,12 but were not borne out in vivo.57,18 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.19,20 Avoiding biofilm development in vivo may depend on minimizing biofouling. Exposing TTs to blood before bacterial contamination pro-

---

**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.

Submitted for Publication: March 31, 2010; accepted May 12, 2010.

Correspondence: Patrick J. Antonelli, MD, Department of Otolaryngology, University of Florida, PO Box 100264, 1600 SW Archer Rd, Gainesville, FL 32610-0264 (Patrick.Antonelli@ent.ufl.edu).

Author Contributions: All authors had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Antonelli and Sampson. Acquisition of data: Sampson. Analysis and interpretation of data: Antonelli, Sampson, and Ojano-Dirain. Drafting of the manuscript: Antonelli, Sampson, and Ojano-Dirain. Critical revision of the manuscript for important intellectual content: Antonelli and Sampson. Statistical analysis: Ojano-Dirain. Obtained funding: Antonelli and Sampson. Administrative, technical, and material support: Antonelli and Ojano-Dirain. Study supervision: Antonelli and Sampson.

Financial Disclosure: Dr Antonelli reported receiving prior grant support and nominal consulting fees on unrelated matters from Medtronic ENT. In addition, he reports having received speaking and writing honoraria from Alcon Labs and WebMD; grant support from Med-El, Kimberly Clark, Sharklet Technologies, ArthroCare, and Alcon Labs; is a consultant for Axogen and ArthroCare; and is on the advisory board of Alcon Labs and Sharklet Technologies.
Funding/Support: This study was supported by a grant from Medtronic ENT, Jacksonville, Florida.

Role of the Sponsors: The study sponsor had no role in the design or conduct of the study and was not involved in the preparation of the manuscript.

Previous Presentation: This study was presented at the American Society of Pediatric Otolaryngology Annual Meeting; April 30, 2010; Las Vegas, Nevada.

REFERENCES