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Prevention of biofilm formation by polyquaternary polymer☆

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ABSTRACT

Objective: Biofilm formation has been linked to device-associated infections in otolaryngology. This study was conducted to determine if a microbicidal polyquaternary polymer, poly diallyl-dimethylammonium chloride (pDADMAC) could prevent biofilm development by pathogens that commonly cause implant infections, *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

Methods and materials: This study was prospective and controlled *in vitro* microbiological study. Polyurethane tubes (20 per treatment) with and without a polyquaternary polymer coating were briefly exposed to plasma or saline, then to *S. aureus* or *P. aeruginosa*. Polyurethane tubes were incubated in growth media. After 4 days, antibiotics were added to kill planktonic bacteria. *S. aureus* or *P. aeruginosa* bacterial counts and scanning electron microscopy (SEM) were performed.

Results: *S. aureus* biofilm counts were reduced by 8 logs on tubes with polyquaternary polymer coating compared to the control tubes, either with plasma (3.67E+01 ± 7.30E+01 vs 1.08E+09 ± 4.81E+08; $P < 0.0001$) or without plasma (3.70E+00 ± 1.10E+01 vs 6.50E+08 ± 2.79E+08; $P < 0.0001$). *P. aeruginosa* biofilm formation was also reduced on tubes with polyquaternary polymer, either with plasma (2.90E+07 ± 1.71E+07 vs 9.16E+08 ± 4.43E+08; $P < 0.0001$) or without plasma (2.50E+07 ± 9.54E+06 vs 3.35E+08 ± 2.18E+08; $P < 0.001$), but the reduction was only 1 log. On control tubes, plasma promoted *S. aureus* (1.08E+09 ± 4.81E+08 vs 6.05E+08 ± 2.79E+08; $P < 0.0001$) and *P. aeruginosa* (9.16E+08 ± 4.43E+08 vs 3.35E+08 ± 2.18E+08; $P < 0.0001$) bacterial counts but not on the tubes coated with polyquaternary polymer.

Conclusions: Incorporation of the microbicidal polyquaternary polymer, pDADMAC, into polyurethane dramatically inhibits *S. aureus* biofilm formation. Further research is warranted to evaluate the efficacy and safety of this technology in otolaryngologic implants.

1. Introduction

Bacterial biofilms have been linked to a variety of otolaryngologic infections [1]. Indwelling and implantable devices such as tracheostomy tubes, airway stents, tympanostomy tubes and cochlear implants are susceptible to bacterial colonization and biofilm formation, which may lead to chronic local inflammation, infection, delayed wound healing, device failure, extrusion, or need for implant removal [2–6]. Bacteria in the biofilm phenotype are tolerant to antibiotic therapy and harder to eliminate due to a slow growth rate, the presence of a protective layer of extra-cellular matrix, and the potential to share antibiotic resistance genes among the bacteria [1]. Because eradication of an established biofilm is difficult to accomplish, several strategies have been developed to make devices resistant to the bacterial attachment and colonization through different surface modifications.

One such modification is the addition of permanent, non-leaching biocides to the material surface. Cationic polymers containing quaternary ammonium salts – also known as polyquats – can be bound covalently to the material surface or integrated in the original polymer used for the medical device [7]. Polyquats have been shown to kill fungi, Gram-positive and Gram-negative bacteria on contact, by penetration of the long cationic polymer into the cell membrane with concurrent membrane disruption [8,9]. These agents are relatively cheap, not susceptible to leaching and degradation, and less likely to induce bacterial resistance compared to antibiotics [10]. The aim of this study was to determine whether the polyquat polymer coating, pDADMAC, could reduce biofilm

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development by *Staphylococcus aureus* and *Pseudomonas aeruginosa*, which are causative microorganisms in a variety of device-related infections in otolaryngology.

2. Methods

2.1. Study design

As this study involved neither human nor animal subjects, it was exempt from institutional review. Individuals performing the quantitative bacterial counts and scanning electron microscopy were blinded to the treatment groups.

A total of 160 polyurethane tubes of identical size, 7-mm long with 2-mm diameter, with and without polyquat polymer coating were used. These were provided by Quick-Med Technologies, Inc. (Gainesville, FL, USA). The proprietary polyquat polymer used was poly diallyl-dimethylammonium chloride, or pDADMAC. Eighty tubes have pDADMAC coating while the other 80 tubes do not have pDADMAC coating. There were a total of 4 treatments for each bacterial strain, divided accordingly among the fluid and bacterial exposure groups (20 per treatment, Table 1).

Each batch included tubes with and without polyquaternary polymer, and were exposed to phosphate-buffered saline (PBS) or undiluted human plasma (from a single patient, donated by a local hospital), to mimic blood contamination that occurs *in vivo*. We have shown that plasma may promote biofilm formation of the bacterial strains used in this study (*S. aureus* ATCC strain 29213 and *P. aeruginosa* strain PAO1) on inert surfaces [11]. The n = 20 reflect technical replicates done on the same day. Bacterial growth was quantified by means of bacterial counts (18 samples per treatment) and SEM (2 samples per treatment). Due to the number of tubes used for each treatment, the experiments for *S. aureus* and *P. aeruginosa* were done on separate days. Study variables (eg, culture duration and sonication time) were optimized through earlier pilot trials.

2.2. Bacterial strains and preparation of the polyurethane tubes

For each bacterial strain, 20 control tubes and 20 pDADMAC-coated tubes were immersed in plasma and 20 control tubes and 20 pDADMAC-coated tubes were immersed in PBS for 5 min. The tubes were then removed and allowed to dry for another 5 min. The tubes were then transferred into a 24-well plate, with one polyurethane tube per well, and equal CFUs (1×10^8 CFU/mL) of *S. aureus* (ATCC strain 29213) or *P. aeruginosa* (strain PAO1) in 1 mL of tryptic soy broth (TSB, MP Biomedicals, Solon, Ohio) was put in each well of pDADMAC-coated and uncoated tubes. These strains have been studied extensively by our group. *S. aureus* is a known middle ear pathogen and *P. aeruginosa* is a known cause of post-tympanostomy tube occlusion (PTOC). Both bacteria readily form biofilms.

The tubes were inoculated with *S. aureus* or *P. aeruginosa* for 5 min. Then, each tube was carefully and aseptically transferred into each well of a new 24-well micro-titer plate and 1 mL of fresh TSB was added into each well. Based on our prior bacterial attachment experiments, the initial bacterial count in each tube was approximately 1×10^4 CFU/mL.

The polyurethane tubes were cultured with *S. aureus* or *P. aeruginosa* in incubator maintained at 37 °C for 4 days, consistent with prior otologic implant biofilm experiments [11–13] and to provide the maximum bacterial challenge to the pDADMAC-coated tubes. Culture media (TSB, MP Biomedicals, Solon, Ohio) was changed daily for 4 days, by carefully aspirating the media in the wells with a Pasteur pipet, taking care not to disturb the tube in each well. After 4 days, gentamicin sulfate, 200 μg/mL (Sigma, St Louis, Missouri), or oxacillin sodium, 10 μg/mL (Fluka, Steinheim, Germany), were added for 24 h to the fresh culture media to eradicate planktonic *P. aeruginosa* and *S. aureus*, respectively. The minimum inhibitory concentration (MIC) of gentamicin and oxacillin against *P. aeruginosa* strain PAO1 and *S. aureus* strain 29213, respectively, were 3.9 μg/mL and 0.24 μg/mL [12]. Thus, the antibiotic concentrations used to eradicate planktonic bacteria for each bacterial pathogen far exceeded the observed MICs for the specific antibiotic.

Biofilm formation was assessed by quantitative bacterial counts and scanning electron microscopy.

2.3. Assessment of biofilm formation by means of quantitative bacterial counts

Following overnight antibiotic treatment, the polyurethane tubes were washed 4 times for 5 min each time 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). Eighteen polyurethane tubes per treatment were used for bacterial counts. The polyurethane tubes were then transferred to 15-mL flip-top conical tubes (Thermo Fisher Scientific, Rochester, New York) containing 3 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 6 min, with four serial 90-s sonication exposures separated by a 60-s rest. After sonication, the tubes were vortexed (setting 8) for 15 s, serially diluted, and spread plated onto tryptic soy agar in triplicate. Plates were incubated for 18–24 h at 37 °C, and the number of colonies were counted manually. Data were expressed as colony-forming units (CFUs) per milliliter.

2.4. Visualization of the biofilms by scanning electron microscopy

Representative polyurethane tubes (2 from each treatment) not processed for bacterial counts were fixed in 1 mL Trumps fixative (1% glutaraldehyde, 4% formaldehyde in PBS) and stored at 4 °C until processed. Specimens were washed 3 times with PBS for 10 min then fixed for 1 h in 1% osmium tetroxide in PBS (Electron Microscopy Sciences, Hatfield, PA). Specimens were washed once with PBS and 3 times with deionized water for 10 min each. Specimens were dehydrated in ethanol series for 10 min each (25%, 50%, 75%, 95%, 100%) and then hexamethyldisilazane (Electron Microscopy Sciences, Hatfield, PA) for 5 min. Specimens were allowed to air-dry overnight. TTs were then cut in half to allow visualization of inner and outer TT surfaces. Specimens were sputter-coated with gold/palladium with argon gas (Desk II sputter coater: Denton Vacuum USA, Moorestown, NJ) for 45 s and stored under vacuum until imaged (Phenom® scanning electron microscope, Nanoscience Instruments, Inc., Phoenix, AZ).

Table 1
Experimental groups, with number of polyurethane tubes tested for each bacterial strain according to exposure condition.[a]

	Control		pDADMAC	
	Plasma	Saline	Plasma	Saline
S. aureus	20	20	20	20
P. aeruginosa	20	20	20	20

[a] The n = 20 reflect technical replicates done on the same day. Bacterial growth was quantified by means of bacterial counts (18 samples per treatment) and SEM (2 samples per treatment). Due to the number of tubes used for each treatment, the experiments for *S. aureus* and *P. aeruginosa* were done on separate days.