Efficacy of Disinfecting Solutions in Removing Biofilms From Polyvinyl Chloride Tracheostomy Tubes

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Objectives/Hypothesis: Bacterial biofilms are prevalent in pediatric tracheostomy tubes (TTs) and are not completely cleared by standard cleaning with gauze and household detergents. We aimed to examine the effectiveness of different disinfecting solutions to remove Staphylococcus aureus (SA) and Pseudomonas aeruginosa (PA) biofilms from TTs.

Study Design: Prospective, controlled, in vitro microbiologic study.

Methods: Uniform coupons obtained from polyvinyl chloride (PVC) pediatric TTs were briefly exposed to human plasma. The samples were incubated in growth media with either PA or SA for 7 days, and total bacterial growth was monitored by media turbidity. Five sets of 18 coupons each were exposed for 5 minutes to one of five different solutions: 2% aqueous chlorhexidine gluconate solution, 0.3% aqueous sodium hypochlorite, Polident denture cleanser, 3% hydrogen peroxide, or preservative-free phosphate-buffered saline (PBS) as a negative control. Biofilm presence was measured with bacterial counts, and surface integrity was assessed with scanning electron microscopy (SEM).

Results: All treatments significantly reduced mean SA counts (P < .001). Sodium hypochlorite and chlorhexidine were more effective than peroxide and Polident. Chlorhexidine, sodium hypochlorite, and peroxide reduced PA counts (P = .001, .001, and .002, respectively), but Polident tabs had no significant effect. SEM revealed preserved TT surface integrity after exposure to all solutions.

Conclusions: Disinfection with sodium hypochlorite or chlorhexidine solutions significantly reduces SA and PA biofilms on PVC TTs. Standard home care of reusable pediatric TTs may be improved by use of these readily available solutions.

Key Words: Bacterial biofilms, tracheostomy tube, disinfection.

INTRODUCTION

Bacterial biofilms represent a significant source of morbidity related to indwelling medical devices. Adult and pediatric tracheostomy tube (TT) systems become colonized with bacterial biofilms, which may be associated with the formation of granulation tissue, tracheal chondritis, airway stenosis, infections of the stoma and lower respiratory tract, and tube occlusion.1 The polysaccharide matrices of biofilms shield the microorganisms from host immune defenses and may serve as a barrier for the adequate cleaning and disinfection of reusable tubes.2

We have previously reported that cleansing with household detergents is not an effective method to clear bacteria that firmly attach to the pediatric tube surfaces.3 Because the tubes are used in direct contact with nonintact skin and respiratory mucosa, chronic colonization of the material surface may be responsible for many of the observed complications. Therefore, investigation of effective and practical methods to disinfect TTs addresses an important clinical need. It also provides further information to refine patient care guidelines, which are now largely based on anecdotal evidence.

The purpose of this study is to evaluate the efficacy of different disinfecting solutions and techniques to remove bacterial biofilms from TT surfaces in vitro, with an emphasis on methods that can be replicated by patients and caregivers at home.

MATERIALS AND METHODS

Specimen Preparation and Biofilm Formation

Circular coupons of identical size (2 mm in diameter) were punched out of pediatric polyvinyl chloride (PVC) TTs (Shiley, Tyco Healthcare Group LP, Pleasanton, CA) and were sterilized with ethylene oxide. Coupons were immersed in 200 μL human plasma for 5 minutes and allowed to dry for 30 minutes. Frozen aliquots of Pseudomonas aeruginosa (PA), strain Rochester, and Staphylococcus aureus ATCC 29213 (SA) were quad-streaked on tryptic soy agar (TSA) plates. PA and SA were chosen for this study because of their common presence on TTs.3 Three to five colonies were picked and grown overnight at 37°C in a tryptic soy broth (TSB) growth medium. The bacteria were transferred to fresh media and grown to early log phase (optical density of 0.1–0.2 at 640 nm). This yielded approximately 108 colony-forming units (CFUs)/mL of both PA and SA, as was previously determined by measuring optical density at 640 nm and interpolating CFUs per milliliter from a predetermined linear
optical density-CFU regression. Coupons were placed in 96-well microtiter plates and incubated in 200 μL of TSB at 37°C for 7 days. Each of the 7 days, the media was replaced with fresh 200 μL of sterile TSB to prevent nutrient depletion.

Disinfection
After 7 days of incubation, the TSB medium was removed, and five sets of 18 coupons each were exposed for 5 minutes to one of five different solutions: 2% aqueous chlorhexidine gluconate solution, 0.3% aqueous sodium hypochlorite, Polident denture cleanser (GlaxoSmithKline Consumer Healthcare, Philadelphia, PA; 2.7 g tablet dissolved in 240 mL of warm sterile water), 3% hydrogen peroxide, or preservative-free phosphate-buffered saline (PBS) as a negative control. Polident tablets are commercially available dissolving tablets marketed as an antibacterial effervescent denture cleaning solution and reported to be highly efficacious for clearing bacteria from dental appliances. After the 5-minute exposure, each sample was rinsed four times with 200 μL of PBS to remove residual disinfectant solution and planktonic bacteria.

Biofilm Analysis
Sixteen coupons from each treatment were transferred to 15-mL conical tubes (Thermo Fisher Scientific, Rochester, NY) containing 1 mL of PBS with 5 ppm of Tween-80 (Fisher Chemical, Fair Lawn, NJ) and serially sonicated (Branson 2510; Branson Ultrasonics, Danbury, CT) for a total of 7.5 minutes, with serial 1.5-minute sonication exposures separated by a 1-minute rest. After sonication, the samples were vortexed for 15 seconds, serially diluted, and plated onto TSA in triplicate. Plates were incubated for 18 to 24 hours at 37°C, and the colonies were manually counted.

Scanning Electron Microscopy
Two nonsonicated coupons from each of the five treatments were transferred to Trump’s solution for fixation. The samples were dehydrated in a graded ethanol series of 25%, 50%, 75%, 95%, and 100% at 10-minute intervals and then hexamethyldisilazane (Electron Microscopy Sciences, Hatfield, PA) for 5 minutes. Specimens were allowed to air dry overnight. Each tube section was then mounted on a scanning electron microscope (SEM) stub and stored in a desiccator until sputter coated with gold and palladium using an argon gas sputter coating unit (Desk II sputter coater; Denton Vacuum USA, Moorestown, NJ) for 45 seconds. The specimens were imaged using a tabletop SEM (Phenom FEI Company, Hillsboro, OR) and examined for the presence of biofilms.

RESULTS
Exposure to all disinfecting solutions was found to significantly reduce mean SA counts compared to controls treated with saline using one-way ANOVA (P < .001). Using Bonferroni’s post hoc test, we found that 0.3% sodium hypochlorite and chlorhexidine were equally effective (P = .81), and both were more effective than hydrogen peroxide (P = .01 and .02, respectively) and Polident (P = .02 and .04, respectively) (Fig. 1). Chlorhexidine, sodium hypochlorite, and peroxide significantly reduced PA counts compared to controls using one-way ANOVA and Bonferroni post hoc test (P = .001, .001, and .002, respectively), while Polident tabs had no significant effect. Sodium hypochlorite and chlorhexidine were both effective in eradicating PA biofilms in this

Treatment Effects on Structural Integrity
The potential caustic effects of sodium hypochlorite on PVC were further investigated by subjecting six groups of 2-mm tube samples to 5-minute soakings one, five, or 10 times. These immersions were done at two different concentrations of sodium hypochlorite (0.3% or 0.6%) followed by rinsing with distilled water. Samples were then prepared for SEM as described. These treated coupons were qualitatively compared to four untreated identical samples by a blinded observer in terms of surface changes demonstrated by cracks, grooves, or peeling of the surface coating. Rigid PVC is generally considered resistant to oxidation by sodium hypochlorite, but the PVC in TTs is combined with phthalate plasticizers to make it soft and pliable. The resistance of this combination to damage by bleach has not been studied yet.

Statistical Analysis
Data were analyzed per bacterial strain using JMP 8 (SAS Institute Inc., Cary, NC) and GraphPad Prism (GraphPad Software, La Jolla, CA). The quantitative bacterial counts were compared between the disinfecting solutions and saline using one-way analysis of variance (ANOVA) followed by Dunnett’s multiple comparison test. Each quantitative bacterial count was also compared against each other using one-way ANOVA. When the result of one-way ANOVA was significant, colony counts were compared against each other using the Bonferroni multiple comparison test. Differences were considered significant for P < .05.
model, with hypochlorite achieving complete eradication of bacteria (Fig. 2).

Review of electron microscopy of SA- and PA-infected coupons after treatment correlated well with the bacterial counts as seen in Figures 3 and 4. We did not observe any fissuring, erosion, peeling or other sign of compromise of the PVC surface in any of the coupons exposed to 0.3% or 0.6% sodium hypochlorite for up to ten 5-minute immersions.

DISCUSSION

This study was designed to investigate simple and reproducible methods to reduce bacterial biofilms on the surface of PVC TT tubes. We demonstrated that both PA and SA biofilms are significantly reduced by a 5-minute immersion in 0.3% hypochlorite or 2% chlorhexidine solutions without damage to the material surface due to bleach. These mature biofilms were allowed to develop over 7 days following exposure to human plasma to replicate the physiologic environment in the trachea with the mild trauma of having a TT placed. The plasma proteins may also serve as attachment site and growth medium for these microorganisms.

It is well known that conventional methods of antimicrobial control are less effective against bacteria in biofilms than planktonic organisms. The slower growth of bacteria in a biofilm allows for greater expression of defensive mechanisms to resist the effects of antibiotics, and the protective layer extracellular matrix creates an environment that favors bacterial attachment and growth. The presence of biofilms in medical devices is associated with chronic disease, pneumonia, failure of re-epithelialization, and other inflammatory states in even the immune-competent host. Other authors have demonstrated that soaking adult TTs in warm water with detergent followed by mechanical cleansing with gauze pads significantly reduced the bacterial load. This method did not prove as effective in pediatric tubes, when recovery of bacteria after cleaning was enhanced by sonication to disperse firmly attached biofilms.

Bleach and chlorhexidine are routinely used microbial disinfectants and were chosen to avoid accelerated material degradation by other methods such as immersion in boiling water or harsher mechanical debridement. The effectiveness of sodium hypochlorite has been evaluated in multiple studies which have shown that sodium hypochlorite in concentrations ranging from 0.1% to 0.5% at various exposure times is adequate for terminal disinfection of reusable medical equipment and eliminates a wide variety of bacteria and
bacterial spores, as well as mycobacteria and viruses. Effective decontamination is only compromised if a significant amount of organic matter is present or where microorganisms are dried to the surface, which highlights the continued need for mechanical cleansing. The concentration used in our study is within a previously reported range and was effective even on dried bacteria attached to a plasma-coated surface. The use of bleach solution has the clear advantages of low price, wide availability, and ease of preparation over chlorhexidine. The commercially available formulations contain 3% to 6% sodium hypochlorite, which may then be mixed with tap water in a 1:5 to 1:10 dilution. The use of bleach may raise concerns about the local and systemic toxicity of the solution, which is defined by the concentration of hypochlorite and the pH of the solution. It is recognized that hypochlorite solutions with concentration higher than 10% are corrosive, but solutions with concentrations of less than 10% are irritants only. Also, liquid household bleach available in the United States has a pH around 11, which is lower than what is considered to be the critical level for tissue corrosive effects of 12.5. Different studies have demonstrated that exposure to household bleach results in only transient effects and no permanent sequelae. Therefore, the concentration we used of 0.3% to 0.6% (5- to 10-fold dilution of commercial bleach) is safe for use in exposed skin and mucosa. Because commercial bleach often contains sodium hydroxide and other alkaline salts or stabilizers, removal of solution residue by flushing in tap water is still recommended.

We assumed that TTs would be changed on a weekly basis and used over a 2- to 3-month period, hence the rationale for testing up to 10 exposures to dilute bleach to assess material integrity. We did not observe significant increases in fissures, cracks, or any other observable damage to the PVC surface on SEM. Previous authors have used a similar technique to study material wear of TTs, supplemented by infrared spectroscopy to detect changes of the chemical bonds of the polymeric chain in PVC, silicone, and polyurethane samples. SEM showed good correlation with spectroscopy results and was an effective tool to investigate degradation, increased brittleness, pores, cracks, and pits on the surfaces and whether those changes facilitate the growth of biofilms.

The in vitro nature of the experiment has limitations. Coupons were briefly exposed only to human plasma. This differs from the presence of mucus, saliva, and moisture in a physiologic environment. Fouling with other organic matter might affect the types of microorganisms present on the TTs as well as impair the

Fig. 4. Scanning electron micrographs of polyvinyl chloride tube coupons cultured with Pseudomonas aeruginosa and exposed to (A) chlorhexidine, (B) bleach, (C) hydrogen peroxide, (D) Polident solution, or (E) saline.
effectiveness of disinfection. Middle ear mucus has been found to not affect PA biofilm development on tympanostomy tubes.\textsuperscript{18} SA and PA are commonly associated pathogens that reside in biofilms, but physiologic multispecies biofilms are more complex than the single-species in vitro biofilms tested.\textsuperscript{4} The increased complexity of a multi-culture-species biofilm results in more variability in the biofilm proteins expressed, possibly changing the structure and vulnerability to treatment.\textsuperscript{13}

CONCLUSION
This study showed that a simple and cost-effective procedure of soaking PVC TTs with mature PA and SA biofilms in dilute bleach or chlorhexidine solutions effectively clears these biofilms. Further in vivo studies are needed to address the role of biofilms in TT complications and to determine if effective interventions to limit the burden of pathogenic bacteria on the tube surfaces and stoma can reduce the morbidity of these devices.

BIBLIOGRAPHY