

Bactericidal effects of silver plus titanium dioxide-coated endotracheal tubes on *Pseudomonas aeruginosa* and *Staphylococcus aureus*

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Purpose: Ventilator-associated pneumonia (VAP) is a nosocomial infection resulting in significant morbidity and mortality. *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Staphylococcus aureus* (*S. aureus*) are pathogens associated with VAP. Silver (Ag) coating of endotracheal tubes (ETTs) reduces bacterial colonization, however titanium dioxide (TiO₂) coating has not been studied.

Methods: Five types of ETT coatings were applied over silica layer: Ag, solgel TiO₂, solgel TiO₂ with Ag, Degussa P25 TiO₂ (Degussa TiO₂), and Degussa TiO₂ with Ag. After ETTs were incubated with *P. aeruginosa* or *S. aureus*; colonization was determined quantitatively.

Results: *Pseudomonas aeruginosa* and *S. aureus* grew for 5 days on standard ETTs. Compared to standard ETTs, *P. aeruginosa* growth was significantly inhibited by solgel TiO₂ with Ag at 24 hours, and by Degussa TiO₂ with Ag at 24 and 48 hours after inoculation. No significant difference in *S. aureus* growth was observed between the control and any of the five coatings for 5 days.

Conclusion: *In vitro*, solgel TiO₂ with Ag and Degussa TiO₂ with Ag both attenuated *P. aeruginosa* growth, but demonstrated no effect on *S. aureus* colonization. Further studies using alternative coating and incorporating UV light exposure are needed to identify their potential utility in reducing VAP.

Keywords: ventilator-associated pneumonia, Degussa titanium dioxide, solgel titanium dioxide, quantitative culture

Introduction

Ventilator-associated pneumonia (VAP) is one of the most common nosocomial infections among intensive care unit patients, which prolongs ventilation and hospitalization, resulting in significant morbidity, mortality and medical costs.¹⁻³ Researchers have developed strategies to prevent VAP, including controlling gastric pH, intratracheal intubation with a high-pressure endotracheal tube (ETT) cuff, continuous subglottic suction to prevent microaspiration, promoting oral hygiene with chlorhexidine, and semi-recumbent positioning.⁴⁻⁹

One strategy to reduce VAP is to treat the ETT with an antiseptic agent to control ETT biofilm formation.^{10,11} A biofilm is an aggregate of a bacterial community attached to a solid surface and encased in an exopolysaccharide matrix, making it resistant to antibiotic penetration.¹² Biofilm formation is most prominent in the distal third of the ETT and can occur as early as sixty hours following endotracheal intubation.¹³

Due to silver's antimicrobial properties, Pacheco-Fowler and colleagues studied chlorhexidine and Ag carbonate impregnated ETTs in an *in vitro* model and observed that bacterial growth of *S. aureus*, *P. aeruginosa*, and other organisms was attenuated.¹⁴ Similarly, Rello et al found that bacterial colonization was delayed for seven days in adults intubated with Ag-coated ETTs compared with standard ETTs,¹⁵ and Kollef and colleagues concluded a statistically significant reduction in the VAP incidence in a randomized multicenter trial.¹⁶

Titanium dioxide is widely used as an antimicrobial agent, but it is not commonly employed in the medical setting. In a process known as photocatalysis, TiO₂ is activated into potent oxidative species when exposed to UV light, with antiviral, antibacterial as well as fungicidal actions.¹⁷ Recent, investigations have suggested that the photocatalytic activity is important in destroying a range of bacteria including *S. aureus* and *P. aeruginosa* in aqueous solutions.¹⁸ Photocatalytic activity also is implicated in the inhibition of biofilm formation on TiO₂ based dental implants.^{19,20} In view of these antimicrobial actions it is plausible that TiO₂-coating of ETTs, potentially in combination with UV light, may sterilize ETTs in patients' airways, thereby reducing the risk of VAP.

We conducted a pilot study of bacterial growth behavior on TiO₂-coated ETTs without UV exposure to evaluate the antimicrobial effect of TiO₂ on polyvinyl chloride (PVC) which is used to manufacture ETTs. Initial experiments examined TiO₂ without UV light exposure for two reasons. First, the Ag and TiO₂ combination has not been studied on PVC material. Second, if the Ag and TiO₂ coating has antimicrobial activity without photocatalysis, this would reduce potential toxicity from UV exposure. The objective of this study was to determine if TiO₂-coated ETTs with or without Ag would reduce bacterial colonization by two common VAP pathogens (ie, *P. aeruginosa* or *S. aureus*) in an *in vitro* model compared with standard ETTs.

Material and methods

Solgel TiO₂ synthesis

Sols of TiO₂ were made using a method adapted from Mills and colleagues.²¹ A solution of 10 mL titanium isopropoxide (AC19470 98+%; Acros Organics, Geel, Belgium) in 2.32 g glacial acetic acid (A465-250; Fisher Chemical, Pittsburgh, PA), was stirred into 60 mL deionized water acidified with 0.59 g concentrated nitric acid (S719721; Fisher Chemical). The reaction solution was then heated rapidly and held at 80°C for 8 hours. The solution was concentrated by heating at 150°C to 11 wt% TiO₂ followed by dilution with ethanol (AC61510; Acros Organics) to obtain a translucent white sol containing 6 wt% TiO₂ sol.

Endotracheal tubes

The inner lumens of the ETTs were first coated with silica (SiO₂) which served as a passivating layer to prevent the TiO₂ from degrading the polyvinyl chloride (PVC) and to improve surface wettability by the subsequently applied TiO₂ coatings. The SiO₂ layer was applied using a nebulizer spray of 15 wt% colloidal SiO₂ in deionized water through the ETT, through a custom-designed nozzle, for 1 minute with an air flow rate of 10 liters per minute. The ETT was then dried with 85°C air for 1 minute.

The respective TiO₂ and Ag treatments are summarized in Table 1. Five types of coatings were applied over the SiO₂ layer (Figure 1): 1) solgel TiO₂, 2) solgel TiO₂ with Ag, 3) Degussa TiO₂ (Evonik Degussa, Parsippany, NJ, USA), 4) Degussa TiO₂ with Ag, and 5) Ag (not shown in Figure 1). Each of the coating solutions A–D had 0.19 wt% TiO₂ on a dry weight basis. Water and ethanol were used as solvents in variable ratios for each solution. Solgel TiO₂ was used for solutions A and B, while C and D solutions consisted of solgel and Degussa TiO₂ in the ratio 20:80 wt/wt. Silver nitrate (AgNO₃) was added to solutions B and D. For solution E, AgNO₃ 0.1 wt% was mixed in ethanol-water (50:50 wt/wt). Two types of TiO₂ coatings were applied; solgel

Table 1 Coating solutions with their respective titanium dioxide and silver treatments

Tube	Solution	TiO ₂ source	Solvent	AgNO ₃ :TiO ₂ (mol%)
1	A	Solgel	Water: Ethanol (60:40)	0
2	B	Solgel	Water: Ethanol (60:40)	5.6
3	C	Solgel: Degussa (20:80)	Water: Ethanol (3:97)	0
4	D	Solgel: Degussa (20:80)	Water: Ethanol (5:95)	5.6
5	E	No TiO ₂	Water: Ethanol (50:50)	0.1wt% AgNO ₃

Abbreviations: Ag, silver; AgNO₃, silver nitrate; Degussa TiO₂, Degussa P25 titanium dioxide; TiO₂, titanium dioxide.

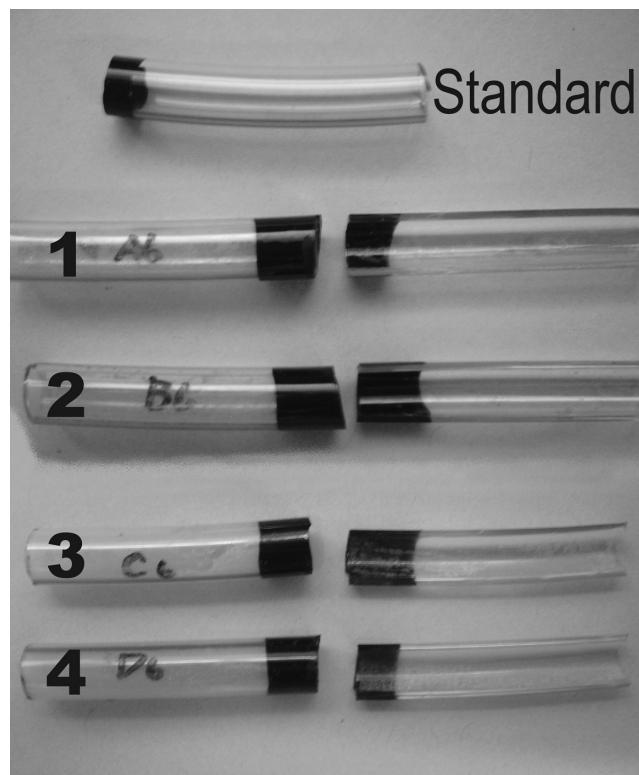


Figure 1 Standard endotracheal tubes were coated with a passivating layer of colloidal silica and served as controls. Five types of coatings were applied over a silica layer; 1. solgel titanium dioxide without silver; 2. solgel titanium dioxide with silver; 3. Degussa titanium dioxide without silver; 4. Degussa titanium dioxide with silver and 5. silver only (not shown).

to increase adherence to PVC for substrate deposition and Degussa TiO₂ for potentially high photocatalytic performance. Since Degussa TiO₂ suspensions had very poor adhesion to the ETTs, they were mixed with a small amount of sols of solgel TiO₂. After TiO₂ coating, the ETTs were dried with 85°C air for 1 minute, followed by further drying in an air-circulating oven at 100°C for 15 minutes, then packed while warm.

Preparation of *P. aeruginosa*

Freeze-dried *P. aeruginosa* (ATCC #25668; American Type Culture Collection, Manassas, VA, USA) was rehydrated with nutrient broth in aliquots to be stored at -80°C. After a loopful of frozen bacteria was plated and incubated, a single colony was inoculated into 4 mL tryptic soy broth (TSB) and incubated overnight at 37°C. The following day, the turbid bacterial broth was vortexed and inoculated. To determine the exact inoculum, 100 µL of bacterial broth was serially diluted with phosphate-buffered saline (PBS), then optical density was measured to obtain 10⁸ colony forming units/milliliter (CFU/mL).

Preparation of *S. aureus*

Frozen *S. aureus* (ATCC #25923) was rehydrated with TSB in the same fashion as *P. aeruginosa*, and stored at -80°C. Frozen *S. aureus* was inoculated into 50 mL TSB in a flask, and incubated overnight at 37°C. Turbid *S. aureus* bacterial broth was transferred to a 50 mL sterile culture tube, centrifuged for 15 minutes, and the supernatant was discarded. A concentrated broth was made by the addition of 1 mL of TSB to the pellet of *S. aureus*. Optical density was measured to obtain 10⁹ CFU/mL for ETT inoculation.

Bacterial inoculation of ETTs

Each ETT was marked at 4 cm intervals for later division into six equal pieces, then immersed in 75% alcohol for 15 minutes to sterilize, rinsed with 20 mL 0.9% sterile saline, and air dried. After sterilization, each ETT was cut into six pieces, sealed on one end with Tegaderm® (3M, St. Paul, MN), filled with either 8 µl of *P. aeruginosa* broth plus 800 µl TSB or 100 µl *S. aureus* broth plus 700 µl TSB, then sealed with Tegaderm. All ETT pieces were placed in a 2 liter dry flask and agitated at 37°C overnight. On the following day, the bacterial fluid was drained from each ETT piece.

Peroxidase assay after coating ETTs

Colorimetric measurement was used to detect whether TiO₂ on ETTs generated peroxidase as part its antimicrobial property. EM Quant Peroxide Test Strips (EMD Chemicals Inc. Gibbstown, NJ, USA) which measure peroxidase between 1–100 parts-per million were used. Tryptic soy broth 800 µL was placed on the TiO₂-coated ETT for 24 hours at 37°C in the dark. Following incubation, the colorimetric strip was dipped into the TSB and the value of peroxidase production was determined.

Observation of bacterial growth

Bacterial inoculated ETT pieces were placed in an incubator to simulate the *in vitro* human trachea with no ambient light, a relative humidity of >90%, temperature of 37°C and at standard atmospheric pressure. Bacterial growth was observed for 24, 48, 72, 96, 120, and 144 hours after inoculation on the standard and five test ETTs. The inner surface of bacteria was collected by washing with 0.5 mL of sterile PBS and swabbing every 24 hours after inoculation. The collected fluid was serially diluted and plated on tryptic soy agar for incubation overnight at 37°C. Colony counts were expressed as CFU/mL, and each experiment was repeated four times.

Statistical analysis

Data were reported as mean ± standard error of mean (SEM). Bacterial colony counts were analyzed using Mann–Whitney *U* test comparing coated ETTs with standard ETTs. A *P*-value of < 0.05 was considered statistically significant.

Results

The coating was slightly opaque using solgel TiO₂ compared with Degussa TiO₂. Samples with Ag-containing coatings appeared dark due to the reduction of AgNO₃ to Ag. The coating was particulate in appearance to the naked eye and peeled off while collecting the bacteria by mechanical force. Peroxidase production was not detected on TiO₂-coated ETTs.

Pseudomonas aeruginosa

Growth peaked at 72 hours and continued for 144 hours on standard ETTs. *Pseudomonas aeruginosa* growth was more robust on Ag-coated ETTs compared to standard ETTs at 120 hours (*P* = 0.04) and 144 hours (*P* = 0.06) despite initial slow growth in the first 24 hours (Figure 2). There was no difference in *P. aeruginosa* growth over 144 hours among standard, solgel TiO₂ and Degussa TiO₂-coated ETTs (Figure 3). Compared to standard ETTs, solgel TiO₂ with Ag inhibited

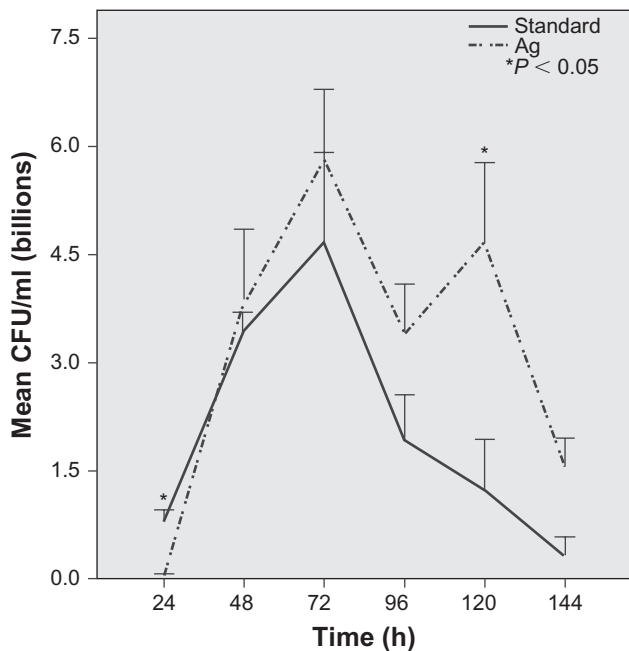


Figure 2 Characterization of *P. aeruginosa* growth on polyvinyl chloride endotracheal tubes over a 144-hour period. Comparison of standard and silver only coated endotracheal tubes. There was less *P. aeruginosa* growth on silver-coated endotracheal tubes at 24 hours (*P* < 0.01), but more growth at 120 hours (*P* = 0.04) and 144 hours (*P* = 0.06) compared to standard endotracheal tubes.

Notes: Mean ± standard error of mean, **P* < 0.05 compared to standard endotracheal tube. *N* = 4.

Abbreviation: CFU, colony-forming units.

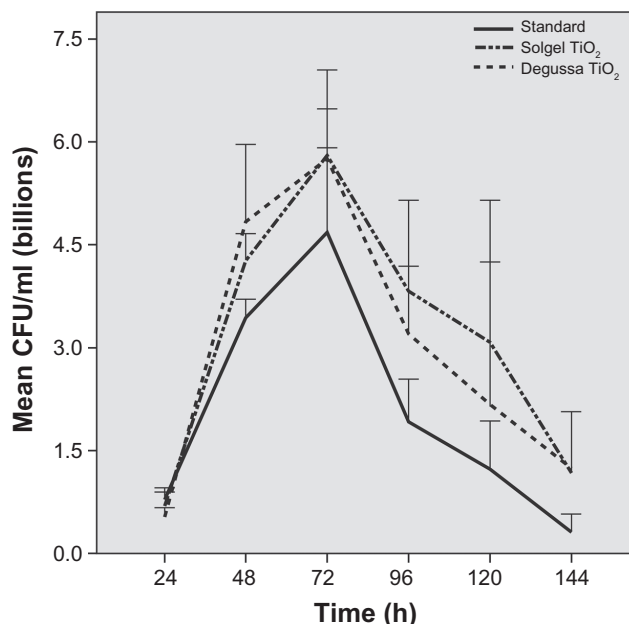


Figure 3 Characterization of *P. aeruginosa* growth on polyvinyl chloride endotracheal tubes coated with titanium dioxide over 144 hour period. Comparison of standard, solgel titanium dioxide and Degussa titanium dioxide endotracheal tubes.

P. aeruginosa growth was not statistically significant at all time points.

Notes: Mean ± standard error of mean, *N* = 4.

Abbreviation: CFU, colony-forming units.

P. aeruginosa growth at 24 hours (*P* = 0.02), and Degussa TiO₂ with Ag inhibited *P. aeruginosa* growth at 24 and 48 hours after inoculation (*P* = 0.02 and *P* = 0.02, respectively) (Figure 4).

Staphylococcus aureus

Growth was observed up to 144 hours. *Staphylococcus aureus* growth peaked at 48 hours after inoculation on both standard and Ag-coated ETTs without a difference over 144 hours (Figure 5). Furthermore, there was also no difference in *S. aureus* growth over 144 hours between standard, solgel TiO₂ and Degussa TiO₂-coated ETTs (Figure 6). Compared to standard ETTs, neither solgel TiO₂ with Ag nor Degussa TiO₂ with Ag showed a difference in *S. aureus* growth over a 144 hour period except solgel TiO₂ with Ag at the 144 hour point after inoculation in which there was a significant attenuation of *S. aureus* growth compared to the standard ETT (Figure 7).

Discussion

Clinically, VAP is diagnosed after patients have been mechanically ventilated for more than 48 hours similar to the duration of bacterial growth in our simulated model. The growth of *P. aeruginosa* was consistent with the findings of other investigators under comparable conditions when monitoring growth in the first 24 hours.^{22,23} An initial suppression of *P. aeruginosa* in Ag-coated ETT was also

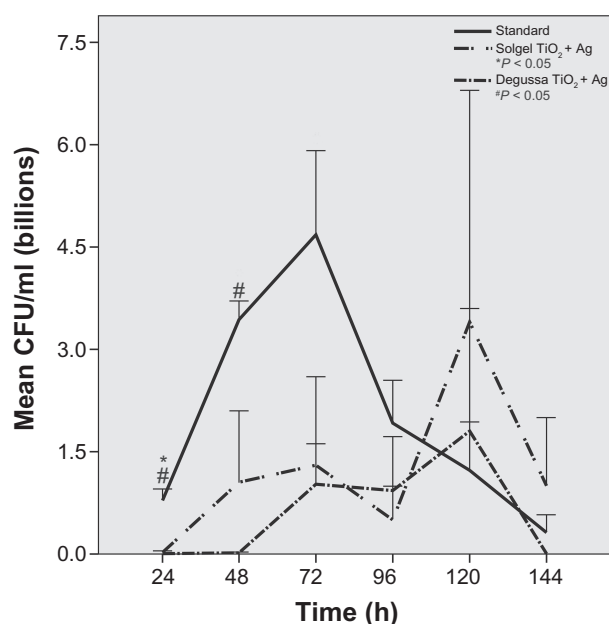


Figure 4 Characterization of *P. aeruginosa* growth on polyvinyl chloride endotracheal tubes coated with titanium dioxide with silver over a 144-hour period. Comparison of standard, solgel titanium dioxide with silver, and Degussa titanium dioxide with silver endotracheal tubes. *P. aeruginosa* growth was inhibited significantly by solgel titanium dioxide with silver at 24 hours and by Degussa titanium dioxide with silver at 24 and 48 hours, respectively.

Notes: Mean \pm standard error of mean; * $P < 0.05$ compared to standard endotracheal tube, # $P < 0.05$ compared to standard endotracheal tube. N = 4.

Abbreviation: CFU, colony-forming units.

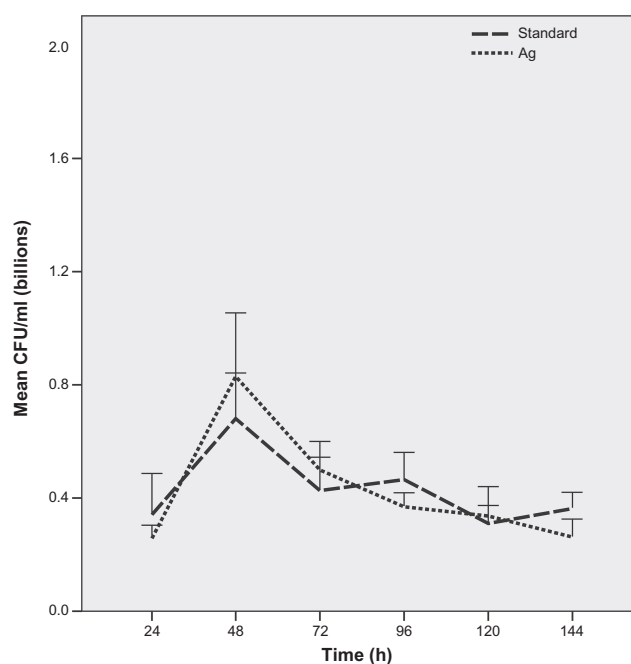


Figure 5 Characterization of *S. aureus* growth on polyvinyl chloride endotracheal tubes coated with silver only over a 144-hour period. Comparison of standard and silver only coated endotracheal tubes. There was no difference in *S. aureus* growth between standard and silver coated endotracheal tubes at any time point over 144 hours compared with standard endotracheal tubes.

Note: N = 4.

Abbreviation: CFU, colony-forming units.

observed in sections of intraventricular tubing impregnated with Ag.²³ Moreover, our experience was similar to results of others who observed an undulating pattern of bacterial growth over a 50 hour period in Ag-coated ETTs.²⁴ The second *P. aeruginosa* growth peak observed in Figure 2 was thought to be an experimental variation due to the small sample size.

A similar growth pattern on Ag-coated ETT was also observed in a study evaluating the growth of *P. aeruginosa* and *S. aureus* on PVC even in the presence of antimicrobials. Both *P. aeruginosa* and *S. aureus* produce a biofilm; its robustness is strain dependent.^{25–27} Biofilm generating species of *S. aureus* exhibit accelerating growth kinetics in the first 6 days in animal models. Furthermore, these strains form microcolonies on catheter substrates that serve as sites of biofilm formation.²⁸ Thus, it is likely that *S. aureus* growth was probably blunted in our model until a glycocalyx developed, then growth proceeded in the presence of nutrients and favorable conditions.

The antimicrobial properties of Ag result from Ag ions binding to bacterial sulphhydryl- or histidyl-containing proteins, which disrupt transmembranous energy metabolism and electrolyte transport systems.²⁹ Because the cell wall composition and thickness vary between gram positive and gram negative bacteria, silver's antimicrobial effect may be

greater in *P. aeruginosa* than in *S. aureus* during the initial phase of bacterial multiplication.

The characteristics of discolored Ag-coated particles were not analyzed in this experiment. However, AgNO₃ is known to undergo photochemical reduction to metallic Ag upon exposure to light as described by the reaction:



This leads to the tiny particles precipitation of Ag that appears black or brown. Future studies are needed to define the specific mechanism involved in the antimicrobial properties of Ag as well as the physiological impact of discolored Ag. Silver nitrate was not expected to react with SiO₂ under ambient conditions, since SiO₂ is quite nonreactive.

Application and treatment of ETTs with these chemicals may also change the surface charge and hydrophobicity, as in this study, thus changing the PVC characteristics from hydrophobic to hydrophilic.

This study demonstrated that the addition of TiO₂ significantly augmented the antibacterial effects of Ag on *P. aeruginosa* during the first 72 hours, but had no effect on *S. aureus*. In our limited study, TiO₂ photocatalysis was not observed as determined by the release of hydrogen peroxide

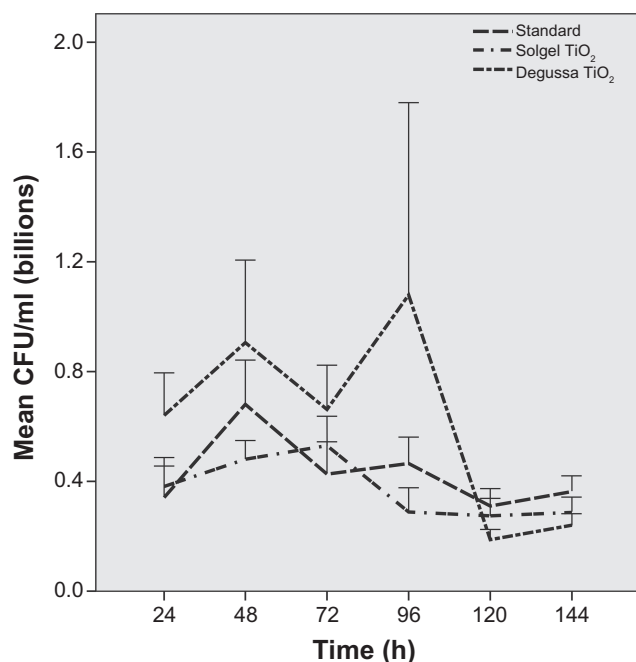


Figure 6 Characterization of *S. aureus* growth on polyvinyl chloride endotracheal tubes coated with titanium dioxide only over a 144-hour period. Comparison of standard, solgel titanium dioxide and Degussa titanium dioxide endotracheal tubes. There was no difference in *S. aureus* growth in the titanium dioxide-coated groups compared to standard endotracheal tubes at any time point.

Note: N = 4.

Abbreviation: CFU, colony-forming units.

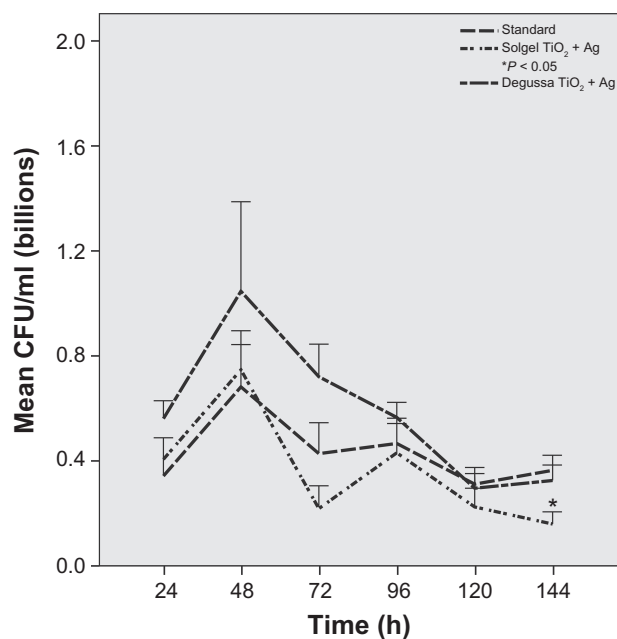


Figure 7 Characterization of *S. aureus* growth on polyvinyl chloride endotracheal tubes coated with titanium dioxide with silver over a 144-hour period. Comparison of standard, solgel titanium dioxide with silver and Degussa titanium dioxide with silver endotracheal tubes. There was no difference in *S. aureus* growth in the titanium dioxide with silver-coated groups compared to standard endotracheal tubes except at 144 hours with solgel titanium dioxide with silver endotracheal tubes.

Note: N = 4.

Abbreviation: CFU, colony-forming units.

as a part of reactive oxygen species. Silver and TiO₂ might have reacted with each other releasing Ag ion, and this reactant might have a synergistic antimicrobial effect even in a dark environment without photocatalysis.

In contrast, Yao showed that application of thin films of Ag and TiO₂ for sterilization purposes on silicone catheters promptly eliminates *S. aureus* within 90 minutes without TiO₂-photocatalysis. They also showed inhibition of growth of *Escherichia coli* (*E. coli*) and *P. aeruginosa*, although they only observed growth for 24 hours.³⁰ Li and colleagues demonstrated that AgNO₃ and TiO₂ pulverized to surgical masks reduced *E. coli* and *S. aureus* by 100%. In Li's study, Ag plus TiO₂ pulverized masks were exposed to UV-C irradiation prior to bacterial inoculation.³¹ *Pseudomonas aeruginosa* behaves very differently in the initial phase of bacterial attachment to the surface compared to *S. aureus*. Rogers and colleagues demonstrated that *S. aureus* multiplies and produces a biofilm on the surface that thickens monotonically, becoming hard during growth and softening during starvation. In comparison, *P. aeruginosa* showed much less reproducible behavior; the organism was loosely adhered and detached from the surface after several hours *in vitro*.³² Until *P. aeruginosa* attachment is established,

this initial motile motion phase may be inhibited or delayed by synergistic bactericidal activity of Ag and either form of TiO₂ as we demonstrated.

Our study has several limitations. First, it was conducted in an *in vitro* model that was designed to replicate the human tracheal environment. However, the results may not be applicable to the *in vivo* setting where other factors may impact the ETT surface such as frequent suctioning, nebulizer therapy or host factors. Second, the concomitant use of Ag and TiO₂ would seem to be marginally effective against VAP if it does not inhibit a common cause of VAP (ie, *S. aureus*). Third, minimizing the particulate surface that facilitates bacterial binding could be effective by reducing the surface area for bacterial adherence and growth. Fourth, the coating, peeled off under mechanical stress, could be dislodged by a suction catheter and produce potentially harmful Ag particles in the human airway and lung parenchyma. Nevertheless, titanium dioxide is known to be safe in humans and is used in such products as cosmetics, toothpaste, and sun screen. Hence, the combination of Ag and TiO₂ needs to be evaluated in a clinical setting. Lastly, this study did not directly evaluate the attractive feature of TiO₂ photocatalysis. The potency of TiO₂ either individually or in combination with Ag needs to

be examined in conjunction with photocatalysis. However, exposing tracheal mucosa to UV light involves potential toxicity. This potential risk and, more importantly, its clinical feasibility, need to be ascertained in future studies.

Conclusion

One strategy to reduce the incidence of VAP is to pretreat ETT with an antiseptic agent to reduce or delay bacterial biofilm formation. The use of TiO₂ with Ag-coated ETTs effectively inhibited *P. aeruginosa* growth, but not *S. aureus* growth, in an *in vitro* model of a human trachea environment.

Disclosures

The authors have no conflicts of interest to disclose in this work.

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