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Antimicrobial-coated endotracheal tubes: an experimental study

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Abstract *Objective:* Antibiotic-resistant bacterial biofilm may quickly form on endotracheal tubes (ETTs) and can enter the lungs, potentially causing pneumonia. In an attempt to prevent bacterial colonization, we developed and tested in an in-vitro study and animal study several antibacterial-coated ETTs (silver sulfadiazine with and without carbon in polyurethane, silver sulfadiazine and chlorhexidine with and without carbon in polyurethane, silver–platinum with and without carbon in polyurethane, chlorhexidine in polyurethane, and rose bengal for UV light). *Design, setting, animals, interventions:* After preliminary studies, silver sulfadiazine in polyurethane (SSD-ETT) was selected among the coatings to be challenged every 24 h with 10^4–10^6 *Pseudomonas aeruginosa*/ml and evaluated at 6 h, 24 h, and 72 h with standard microbiological studies, scanning electron microscopy, and confocal scanning microscopy. Subsequently, eight sheep were randomized to receive either a SSD-ETT or a standard ETT (St-ETT). After 24 h of mechanical ventilation, standard microbiological studies were performed together with scanning electron microscopy and confocal microscopy. *Measurements and results:* In the in-vitro study SSD-ETT remained bacteria-free for up to 72 h, whereas St-ETT showed heavy *P. aeruginosa* growth and biofilm formation ($p < 0.01$). In sheep, the SSD-ETT group showed no bacterial growth in the ETT, ventilator tubing, and lower respiratory tract, while heavy colonization was found in the St-ETT ($p < 0.01$), ventilator tubing ($p = 0.03$), and lower respiratory tract ($p < 0.01$). *Conclusion:* This study describes several effective and durable antibacterial coatings for ETTs. Particularly, SSD-ETT showed prevention against *P. aeruginosa* biofilm formation in a 72-h in-vitro study and lower respiratory tract colonization in sheep mechanically ventilated for 24 h.

Keywords Endotracheal tube · Mechanical ventilation · Bacterial biofilm · Ventilator-associated pneumonia · Silver sulfadiazine

Introduction

Nosocomial pneumonia is one of the leading causes of morbidity and mortality in hospitalized patients [1, 2]. While a number of factors contribute to the increased risk of nosocomial pneumonia in patients in the intensive care unit, mechanical ventilation using an endotracheal tube (ETT) represents one of the greatest risks [3–5].

Recent studies showed that antimicrobial-coated ETT may be used to lower lung colonization, claiming to lower the incidence of ventilator-associated pneumonia (VAP) [6–14]. In the design of antimicrobial-impregnated biomaterials, both the selection of the type and the rate of release of the antimicrobial agent from the medical device are important. Ideally, the selected antimicrobial agent should possess a lasting broad-spectrum antimicrobial activity and a low degree of bacterial resistance. The widespread occurrence of antibiotic resistance is alarming [15, 16]; hence, an interest has emerged in the use of medical devices coated with nonantibiotic antimicrobial agents.

We explored several biomaterials and fabricated ten different coatings for ETT. After testing the tubes for effectiveness and feasibility, we selected the coating with silver sulfadiazine (SSD-ETT). In an in-vitro experiment, we challenged SSD-ETT and non-coated ETT with *Pseudomonas aeruginosa* to: (a) compare the bacterial growth rate; (b) determine the ability of silver sulfadiazine to prevent bacterial attachment to ETT; and (c) determine the ability of silver sulfadiazine to kill pathogenic bacteria. Secondly, we tested SSD-ETT in sheep to: (a) assess the bactericidal effects of the SSD coating in the ETT and throughout the ventilator circuit; (b) measure the reduction in bacterial colonization of the lungs; and (c) investigate local and systemic side effects.

Materials and methods

ETT coatings: materials and fabrication

In a laboratory at the US National Institutes of Health, Bethesda, Maryland, we fabricated ten bacteriostatic and bactericidal ETT coatings: (1) chlorhexidine in polyurethane, (2) silver sulfadiazine in polyurethane, (3) silver sulfadiazine and chlorhexidine in polyurethane, (4) silver sulfadiazine and carbon in polyurethane, (5) silver sulfadiazine chlorhexidine and carbon in polyurethane, (6) silver in polyurethane, (7) silver and carbon in polyurethane, (8) silver–platinum in polyurethane, (9) silver–platinum and carbon in polyurethane, and (10) rose bengal for UV light (see Fig. 1).

Silver sulfadiazine and chlorhexidine

The silver salt of sulfadiazine with or without chlorhexidine was developed recently. It is widely used to coat

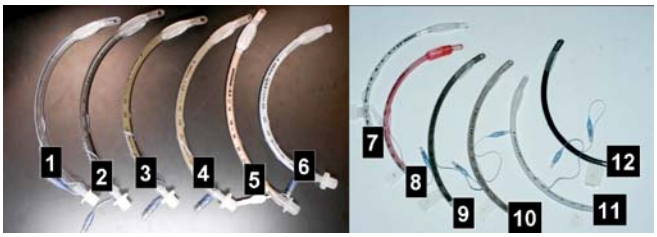

Fig. 1 Coated endotracheal tubes. From *left* to *right*: 1, standard non-coated ETT; 2, silver sulfadiazine chlorhexidine and carbon in polyurethane; 3, silver sulfadiazine and carbon in polyurethane; 4, silver sulfadiazine and chlorhexidine in polyurethane; 5, silver sulfadiazine in polyurethane; 6, chlorhexidine in polyurethane; 7, standard non-coated ETT; 8, rose Bengal for UV light; 9, silver and carbon in polyurethane; 10, silver in polyurethane; 11, silver–platinum in polyurethane; 12, silver–platinum and carbon in polyurethane

medical devices to prevent catheter-related infections and is currently the treatment of choice for burn wounds, as it has activity against gram-negative and gram-positive bacteria, fungi, protozoa, and certain viruses. However, the mechanism of silver sulfadiazine and chlorhexidine's antibacterial action has not been fully elucidated. After exposure, structural changes occur in the bacterial cell membrane, such as distortion and enlargement of the cell and weakening of the membrane. Silver sulfadiazine molecules dissociate, and the silver moiety enters the cell wall, attaches to the DNA, and prevents bacterial cell proliferation. Chlorhexidine alters the cell membrane sufficiently to permit the efflux of nitrogen bases, nucleotides, and nucleosides and facilitate entry of sulfadiazine molecules [17–19].

We coated the lumen of ETT with chlorhexidine in polyurethane; silver sulfadiazine in polyurethane; silver sulfadiazine and chlorhexidine in polyurethane; silver sulfadiazine and carbon in polyurethane; and silver sulfadiazine, chlorhexidine, and carbon in polyurethane (Fig. 1, coated ETT nos. 2–6)

Oligodynamic iontophoresis

One of our early research paths was directed towards electrically injecting metal ions (silver) into solution. This has been shown to reduce bacterial colonization 15- to 100-fold in both bench-top and animal experiments. Bactericidal iontophoretic polymers can be designed to release silver ions when moistened with body fluids in the presence of silver and platinum powder. When the composite material is placed in contact with or immersed in an electrically conductive medium, such as saline, blood, or urine, or mucus, the metal powder becomes an array of small electrodes. Specifically, each metal granule embedded in the base material becomes either an anode or a cathode. Microbial growth is impaired through release of silver ions,

with the generation of electric current from 1 to 400 μA. This range of current does not cause localized cell necrosis and is below the sensory or pain threshold. The amounts of carbon, silver, and platinum powder, their ratios, their particle size and the permeability of the polymer composition all affect the rate of silver ion release [20].

We coated the lumen of ETT with silver in polyurethane; silver and carbon in polyurethane; silver–platinum in polyurethane; and silver–platinum and carbon in polyurethane (Fig. 1, coated ETT nos. 9–12).

Photodynamic therapy

Rose bengal is a perhalogenated fluorescein derivative that is among the most efficient known producers of singlet oxygen. Activation with UV light causes singlet oxygen production and photosensitization. Rose bengal has been widely used in photodynamic therapy of tumors, to inactivate viruses, gram-positive bacteria, and protozoa, and to produce photohemolysis, and to induce occlusion of blood vessels in a procedure called photothrombosis [21]. We coated the lumen of ETT with rose bengal (Fig. 1, coated ETT no. 8). During mechanical ventilation, a probe was connected to an UV-visible light source and introduced inside the ETT.

Coating selection

After having selected the antimicrobial agents and concentrations, we tested the efficacy and safety of the variously coated ETT in repeated in-vitro and animal studies over a period of approximately 2 years. All of them showed bacteriostatic and bactericidal effects [11, 22–24].

However, we selected the silver sulfadiazine in polyurethane coating for further investigation, because:

a) SSD is widely and safely used in the clinical setting (i. e., cream preparation, IV catheters, urinary catheters, prostheses).
b) Unlike UV light, SSD-ETT do not require extra care.
c) The SSD coating is very smooth and resistant to torque.
d) At extubation after prolonged mechanical ventilation in sheep (up to 7 days of intubation) the SSD coating appeared to maintain its characteristics on visual inspection and on microscopy (Fig. 2a, b).
e) One year after the coating process, the SSD coating retained antibacterial properties in repeated studies. Electron microscopy and confocal laser microscopy showed no morphological differences from new, unused SSD-ETT.

The coating procedure

We prepared a dispersion of 53 g of silver sulfadiazine, and 22.5 g of polyurethane (BioSpan) in 210 ml of *N*, *N*-dimethylacetamide. We inserted a standard 8-mm tracheal tube (Lo-Contour TM, Mallinckrodt, St. Louis, USA) into a hollow transparent acrylic tube, to keep the ETT straight. With the plastic tube positioned vertically, we immersed the ETT tip into the dispersion, rapidly aspirated the dispersion up to the level of the connector piece, and then let the ETT drain for 2–4 s. Then we placed the transparent plastic tube with the ETT horizontally into a rotating device, through which a stream of air was gently passed to dry the dispersion. After 12 h, the coated ETT was removed and sterilized with ethylene oxide gas. Pictures of the lumen of the SSD-ETT are shown in Fig. 2.

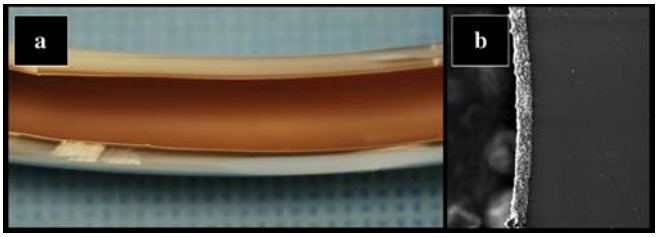

Fig. 2 Silver sulfadiazine in polyurethane coated ETT. **a** Lumen of a SSD-ETT after 7 days of intubation and mechanical ventilation in sheep. The coating looks like the new, never-used coated SSD-ETT. **b** Scanning electron microscopy: cross-section of the same tube

In-vitro study

In a set of experiments (six replicates), 10^4–10^6 *P. aeruginosa*/ml in biofilm medium were placed in the lumen of SSD-ETT and standard non-coated ETT (St-ETT). Strain PaO1 containing the GFP-plasmid (pMRP9-1 carbenicillin resistant) [25] was used to: (a) determine the ability of the silver sulfadiazine coating to prevent bacterial attachment and ETT-biofilm formation and (b) compare bacterial growth rates in biofilm medium.

For the growth medium, carbenicillin powder was diluted to 150 μg/ml using 1% Trypticase Soy Broth solvent. This was used to dilute *P. aeruginosa* PA01 to 10^5–10^7 cells/ml. St-ETT and SSD-ETT were clamped 1 cm proximal from the inflatable cuff and partially filled with 8 ml of freshly prepared biofilm medium. Tubes were clamped 2 cm from the ETT connector piece and incubated at 37 °C with mild shaking.

The bacterial challenge was stopped at 6, 24, and 72 h. Bacterial count of ETT broth was calculated using standard microbiology methods for bacterial quantification counts. Scanning electron microscopy of the ETT lumen was performed to assess the biofilm and the thickness of secretions. Using aseptic technique, a 1-cm-long cross section of ETT was excised and placed in a sterile vial filled with 2.5% glutaraldehyde, and stored at 4 °C for scanning electron microscopy (SEM) and confocal laser scanning microscopy (CLSM) [11].

Animal laboratory study

The study was conducted and approved by the National Institutes of Health.

The SST-ETT animal study was performed in sheep to assess both (a) prevention of bacterial colonization of the respiratory tubing and of the lower respiratory tract and (b) local and systemic side effects in an animal model.

This 24-h study involved eight young female Dorset sheep. Sheep were randomized to receive either a standard ETT ($n = 4$) or an ETT internally coated with silver sulfadiazine in polyurethane ($n = 4$), and were mechanically ventilated for 24 h. The number of sheep for each group was calculated based on the results of our previous study on coated ETT in animals mechanically ventilated for 24 h. The protocol, preparation, and monitoring of the animal study were described previously [11].

At autopsy, the thorax was opened using strict aseptic techniques, and the lungs were exposed, excised, and weighed. Tissue samples were collected for quantitative culture from each lobe and the lobar bronchi. A total of 12 tissue samples weighing approximately 50 mg each were taken: five samples from the five lobes of the lungs, five samples from the five corresponding lobar bronchi, one sample from the trachea 2 cm above the carina, and one sample from the middle part of the ETT (Fig. 3a). The oral cavity was sampled at the beginning of the study. The trachea and the larynx were opened through a longitudinal anterior-midline–incision up to the carina for visual inspection of the mucosa and of the ETT. The trachea was excised and sent for microscopic study. All tissue/mucus and fluids were sent for quantitative and qualitative aerobic cultures using standard bacteriologic techniques. All indwelling devices were cultured.

The internal lumen of the ETT was sampled 10–12 cm from the ETT connector piece every 8 h (four samples) for bacterial growth using a cotton culture swab.

Just before the end of the study, we sampled the air filter between the ventilator and the humidifier, the water from the humidifier, the inspiratory and expiratory lines of the mechanical ventilator approximately 10–12 cm from the ETT connector piece, and the expiratory line condensate water trap (Fig. 3b).

Statistical analysis

We used the Wilcoxon (Mann–Whitney) rank sum test for group comparisons of continuous variables. Fisher's exact

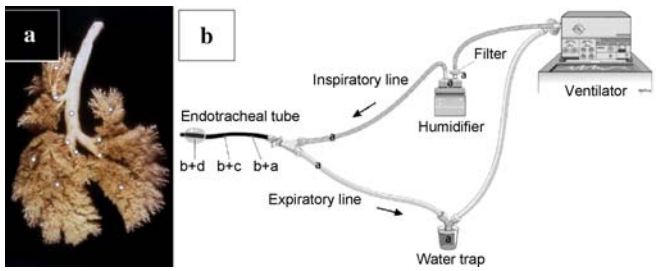

Fig. 3 Animal study: sample sites. **a** Silicon rubber cast of sheep lungs. *White circles* indicate sites from which samples were taken upon autopsy for microbiological studies: trachea, five bronchi, and five lobes of the lungs (from Panigada et al., Crit Care Med 2003; 31:729–737). **b** Ventilator circuit of the sheep study, and sample sites: *a*, Swabs from the air filter, humidifier, inspiratory lines, ETT, expiratory lines, and water trap; *b*, ETT biofilm scraped for light microscopy studies; *c*, Secretions from inside the ETT for bacterial culture (CFU/g); *d*, Two rings of the ETT were cut, one for confocal scanning laser microscopy studies and one for scanning electron microscopy [11]

Table 1 In-vitro study: scanning electron microscopy (*SEM*), confocal laser scanning microscopy (*CLSM*) and microbiology findings in ETT challenged with *P. aeruginosa* after 6 h, 24 h, and 72 h

	6 h		24 h		72 h	
	SSD-ETT	St-ETT	SSD-ETT	St-ETT	SSD-ETT	St-ETT
SEM						
Absence of bacteria	×		×		×	
Adhesion of bacteria		×				
Formation of microcolonies				×		
Confluent colonies						×
Thickness (μm)	0	0–2	0	10–30	0	15–50
CLSM						
Absence of bacteria	×		×		×	
Adhesion of bacteria		×				
Formation of microcolonies				×		
Confluent colonies						×
Thickness (μm)	0	0–5	0	20–50	0	30–70
Microbiology						
Median (cfu/ml)	0	1.2×10^6	0	3.4×10^7	0	2.9×10^6
Range (cfu/ml)	0–0	3.1×10^4–2.4×10^7	0–3.5×10^7	5.7×10^5–3.2×10^9	0–0	5.4×10^5–5.0×10^7
Colonized-ETT ($n = 6$)	0	6	2	6	0	6
p-value *	< 0.01		0.06		< 0.01	

* *p*-values calculated using Fisher's Exact test: SSD-ETT vs. St-ETT

test was used for the analysis of categorical variables. A p-value < 0.05 was considered statistically significant. All tests were two-sided. We performed all analyses with the Stata statistical package (Stata, College Station, TX; release 8.0).

Results

In-vitro study

SEM revealed bacterial adhesion on the St-ETT polyvinylchloride at 6 h (Fig. 4a), formation of microcolonies at 24 h, and uniform protein-like deposits at 72 h (Fig. 4b) (thickness of secretions on the lumen of St-ETT ranged from 0 μm to 70 μm; Table 1). No bacteria or secretions were detected at any time on SSD-ETT (Fig. 4c, d; Table 1). The culture broth was always heavy colonized in the St-ETT (bacterial growth during the 72 h study period ranged from 3.1×10^4 to 3.2×10^9 cfu/g), while in the SDD-ETT bacteria were present only in two samples after 24 h (bacterial growth during the 72 h study period ranged from 0 to 3.5×10^7 cfu/g) (Table 1).

Animal study

All study animals were healthy upon enrollment, based on clinical findings, laboratory data, and chest X-ray during 24 h of mechanical ventilation. Intubation was successful at the first attempt in all sheep. The PaO_2/FiO_2 ratio was greater than 400 at all times in all sheep. No fever, purulent secretions in ETT, abnormal leukocyte counts, or changes on chest radiographs were observed. At autopsy, no gross abnormalities were identified in the tracheal mucosa.

In the St-ETT group, the lower respiratory tract and ventilator tubing were extensively colonized (lower respiratory tract colonization ranged from 5.0×10^5 to 5.5×10^8 cfu/g). No bacterial colonization was detected throughout the lower respiratory circuit, and ventilator tubing (lower respiratory tract colonization ranged 0–0 cfu/g, $p < 0.01$ vs. St-ETT) in sheep intubated with the SSD-ETT (Table 2).

Microscopic studies showed a thick and dense secretion layer covering the lumen of the St-ETT (range 50–750 μm, bacterial colonization 5.0×10^6–3.5×10^8 cfu/g) (Table 2, Fig. 5a), and at higher magnification accumulations of bacteria, white blood cells, and red blood cells could be easily identified (Fig. 5b). On SDD-ETT, the mucus layer was much thinner, ranging from 0 to 450 μm, and no bacteria were observed (bacterial colonization 0–0 cfu/g, $p < 0.01$ vs. St-ETT) (Table 2; Fig. 5c, d). The most common aerobic bacteria found in the oral secretions were α-hemolytic*Streptococcus* spp., *Moraxella* spp., *Pasteurella* spp., and *Staphylococcus aureus*; in addition to those bacteria, from the lower respiratory system of the Et-ETT group we cultured *Kleb-*

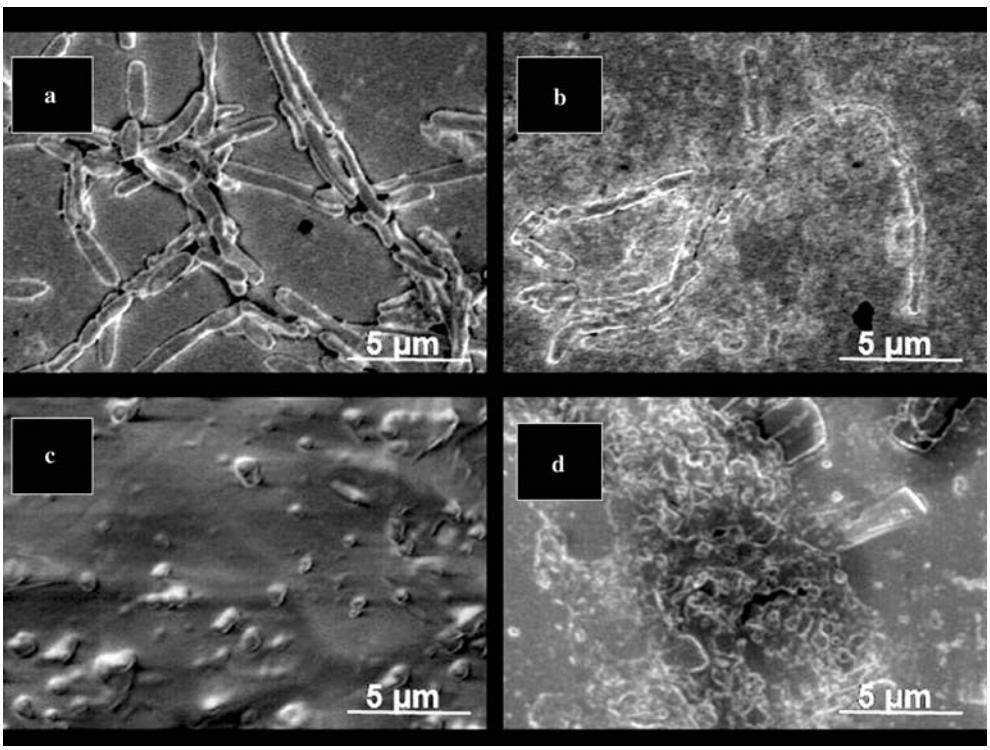

Fig. 4 In-vitro study: SEM micrographs. ETT samples were imaged with scanning electron microscopy. **a** After 6 h of challenge with *P. aeruginosa*, bacteria were seen to adhere to the polyvinylchloride lumen of the St-ETT. **b** After 72 h, a thick biofilm covered the entire tube surface. A chain of bacteria can be seen emerging from the thick biofilm. **c, d** No bacteria were seen to adhere at any time to the coated surface of SSD-ETT (**c** after 6 h; **d** after 72 h). The silver sulfadiazine coating has a granular appearance

Table 2 Animal study: microscopy and bacteriology findings in mechanically ventilated sheep intubated with SSD-ETT and St-ETT

	SSD-ETT	St-ETT	*p*-value
SEM-CSLM			
Thickness of secretion layer, min min–max, μm	25, 0–100	100, 50–200	
Thickness of secretion layer, max min–max, μm	250, 150–450	685, 650–750	
Well-defined biofilm architecture ($n=4$)	0	0	1.00 *
presence of bacteria ($n=4$)	0	3	0.14 *
Presence of red cells ($n=4$)	1	2	1.00 *
Presence of white cells ($n=4$)	1	3	0.49 *
Microbiology			
Tissue biopsy			
Trachea			
Median (cfu/ml)	0	3.8×10^8	< 0.01 **
Range (cfu/ml)	0–0	$5.0 \times 10^5 - 4.5 \times 10^8$	
Bronchi			
Median (cfu/ml)	0	5.8×10^7	< 0.01 **
Range (cfu/ml)	0–0	$9.0 \times 10^6 - 5.5 \times 10^8$	
Lungs			
Median (cfu/ml)	0	7.3×10^7	< 0.01 **
Range (cfu/ml)	0–0	$4.5 \times 10^6 - 4.0 \times 10^8$	
ETT			
Median (cfu/ml)	0	1.3×10^8	< 0.01 **
Range (cfu/ml)	0–0	5.0×10^6–3.5×10^8	
Cotton swab			
Inspiratory line ($n=4$)	0	4	0.03 *
Expiratory line ($n=4$)	0	4	0.03 *
Humidifier ($n=4$)	0	4	0.03 *
Water trap ($n=4$)	0	4	0.03 *

* *p*-values were calculated using Fisher's exact test; ** *p*-values were calculated using Wilcoxon (Mann–Whitney) rank sum test; The most common aerobic bacteria detected included: α-hemolytic *Streptococcus* spp. (not *Streptococcus pneumoniae*), *Klebsiella pneumoniae, Moraxella* spp*, Pasteurella haemolytica*, *Pasteurella multocida*, *Pasteurella* spp, *P. aeruginosa*, *Staphylococcus aureus*

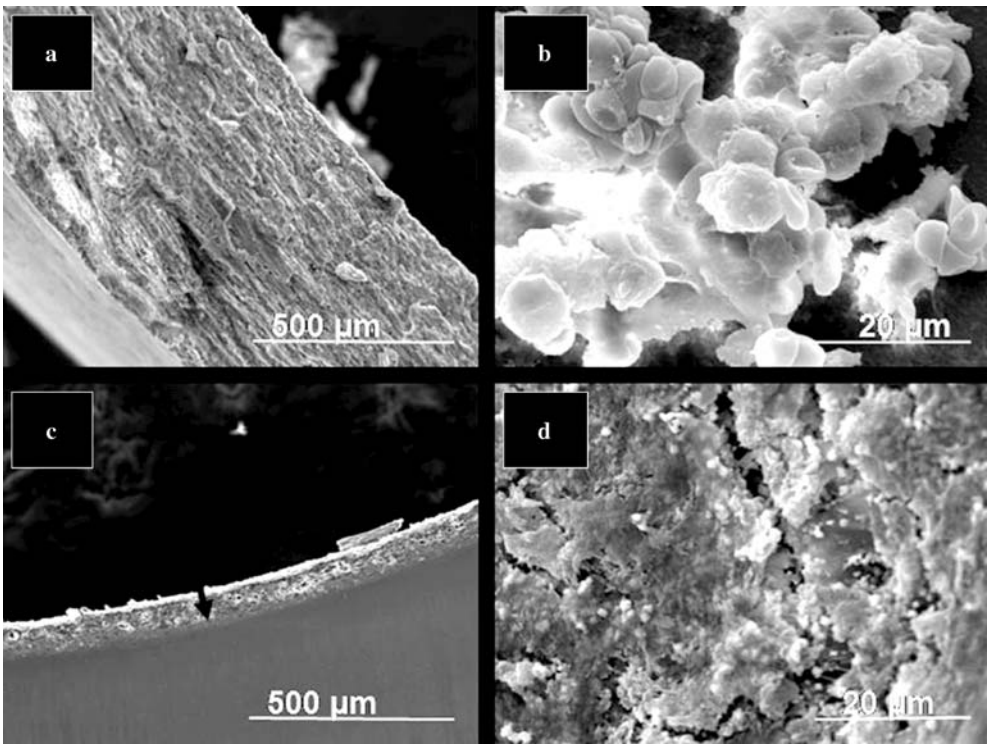

Fig. 5 Animal study: SEM micrographs. ETT samples were imaged with scanning electron microscopy. **a** Note the thick (almost 700 μm) deposits on the lumen of St-ETT of sheep that were intubated and mechanically ventilated for 24 h. **b** At higher magnification, red cells can be easily recognized. **c, d** No secretions accumulated on SSD-ETT. The *arrow* (**c**) shows the thickness of the coating (approximately 40 μm)

Table 3 Coated andotracheal tube studies

Publication	Materials for the ETT coating	Study-length	Conclusion of the study
In-vitro studies			
Hartmann et al. [6]	Silver based	50 h	Silver-coated ETT showed a significant inhibition of growth of *Pseudomonas aeruginosa* in the continuously contaminated and mechanically ventilated oropharynx–larynx–lung model. Silver-coated ETT thus might be helpful in reducing VAP.
Jones et al. [7]	Hexetidine-impregnated ETT	8 h	Based on the impressive microbial anti-adherence properties and durability of the surfactant coating on PVC following dip coatings, it is proposed that these systems may usefully reduce the incidence of ventilator-associated pneumonia when employed as luminal coatings of the ETT.
Balazs et al. [8]	Chemical modification of ETT-PVC with sodium hydroxide and silver nitrate solutions	72 h	The chemical modifications using NaOH and AgNO(3) wet treatments completely inhibited bacterial adhesion of 4 strains of *P. aeruginosa* to both native and oxygen-pre-functionalized PVC, and efficiently prevented colonization over 72 h.
Pacheco-Fowler et al. [14]	ETTs impregnated with chlorhexidine and silver carbonate	5 days	Antiseptic ETTs prevented bacterial colonization in the airway model and also retained significant amounts of the antiseptic.
Chaiban et al. [9]	Gentian violet and chlorhexidine	3 weeks	Coated ETT impregnated using an instantaneous dip method, were shown to have broad-spectrum activity, prolonged antimicrobial durability and high efficacy in inhibiting adherence of organisms commonly causing nosocomial pneumonia. Furthermore, these coated devices were shown to be non-cytotoxic.
Animal studies			
Olson et al. [10]	Silver based	96 h	These results suggest that the silver coating of ETTs may delay the onset of and decrease the severity of lung colonization by aerobic bacteria.
Berra et al. [11]	Silver sulfadiazine and chlorhexidine	24 h	Coated ETTs induced a nonsignificant reduction of the tracheal colonization, eliminated (seven of eight) or reduced (one of eight) bacterial colonization of the ETT and ventilator circuits, and prevented lung bacterial colonization.
Clinical trial			
Rello et al. [13]	Silver based	up to 32 days	In this prospectively planned, preliminary analysis, the ETT coated was feasible and well tolerated. Larger studies are needed to determine whether delayed colonization, reduced colonization rate, and decreased bacterial burden will decrease the incidence of VAP.

siella pneumoniae, Pasteurella haemolytica, Pasteurella multocida, and *P. aeruginosa*.

Discussion

The incorporation of an antimicrobial agent within the constituent polymer of a medical device is an accepted method to decrease the incidence of device-associated infection. This approach may reduce microbial adherence to the biomaterial by virtue of an antimicrobial surface, as well as by the release of drug into the surrounding medium in quantities sufficient to achieve microbial killing. The inclusion of antimicrobial agents to the component polymers of medical devices has resulted in improved outcomes when using devices such as central venous catheters, urinary catheters, bone cements, cerebrospinal fluid shunts, and continuous peritoneal dialysis catheters [26–29].

Therefore, prevention of bacterial colonization of the polyvinyl chloride (PVC) ETT has been suggested to lower contamination of the lower respiratory system, and thus the incidence of VAP. Many investigators have recently focused their research on novel materials to coat the ETT to prevent bacterial colonization. Table 3 summarizes some of those studies.

In 1999, Hartmann et al. published the first study on coated ETT [6]. The ETT was covered under vacuum with 0.15- to 0.25-μm-thick silver films after pre-coating with different precious metals. They developed an oropharynx–larynx–lung model which was continuously contaminated with *P. aeruginosa* and which for the purpose of clinical simulation was mechanically ventilated for a period of 50 h. Coated ETTs showed significant reduction of bacterial counts in the oropharynx–larynx model throughout. In 2001, Jones et al. conducted an in-vitro study with hexetidine-impregnated PVC ETT [7]. PVC emulsion was cured in the presence of hexetidine. The purpose of the study was to examine hexetidine-impregnated PVC ETT biomaterials with respect to their tensile, surface, and drug release properties, and also their resistance to the adherence of clinical isolates of *Staphylococcus aureus* and *P. aeruginosa*. ETT PVC containing hexetidine significantly lowered the number of adherent viable bacteria. In a fascinating study in 2004, Balazs et al. incorporated monovalent silver into the PVC [8]. The antiadhesive and antibacterial properties of this new material were tested on *P. aeruginosa*. The chemical modification consisted of a radiofrequency-oxygen glow discharge prefunctionalization, followed by a two-step wet treatment in NaOH and $AgNO_3$ solutions. The creation of silver salt on native PVC resulted in an ultrahydrophobic surface. The chemical modifications using NaOH and $AgNO_3$ wet treatments completely inhibited bacterial adhesion of four strains of *P. aeruginosa* and efficiently prevented colonization for 72 h. In the same year, Pachego-Fowler et al. published a study employing chlorhexidine and silver carbonate-coated ETT [14]. The antiseptic ETT were compared with non-coated ETT to evaluate the potential effectiveness of impregnating ETT with antiseptic to reduce colonization of methicillin-resistant *S. aureus*, *P. aeruginosa*, *Acinetobacter baumannii*, and *Enterobacter aerogenes*. In an in-vitro model the authors showed that antiseptic ETT significantly decreased or prevented bacterial colonization for 5 days in the tracheal model and retained substantial amounts of the antiseptic agents. There are only two animal studies exploring the benefits of coated ETT. We showed that silver sulfadiazine and chlorhexidine in polyurethane decreases bacterial colonization of the lower respiratory tract of sheep and ventilator circuit after 24 h of mechanical ventilation [11]. In a recent study, Olson et al. explored the benefits of hydrogel silver-coated ETT (C.R. Bard) on 12 dogs challenged with *P. aeruginosa* [10]. They found that silver coating of ETT delayed the appearance of bacteria on the inner surface of the ETT and decreased lung bacterial colonization.

In our laboratory, we developed 10 different coatings for ETTs: (1) chlorhexidine in polyurethane, (2) silver sulfadiazine in polyurethane, (3) silver sulfadiazine and chlorhexidine in polyurethane, (4) silver sulfadiazine and carbon in polyurethane, (5) silver sulfadiazine chlorhexidine and carbon in polyurethane, (6) silver in polyurethane, (7) silver and carbon in polyurethane, (8) silver–platinum in polyurethane, (9) silver–platinum and carbon in polyurethane, and (10) rose bengal for UV light. We decided not to use antibiotics to coat the ETT, because the indiscriminate use of antibiotics may facilitate rapid dissemination of multiresistant bacteria. Each of the 10 coatings showed bactericidal or bacteriostatic properties in vitro and in animal studies. While all these coatings are effective to prevent bacterial colonization, we decided to select only one coating for further testing. Our selection criteria were: (1) the safety for the patient and (2) the feasibility of using the tubes in the hospital. Using PubMed and the US Food and Drug Administration database, we learned that chlorhexidine occasionally causes immediate systemic hypersensitivity reaction [30]. Carbon powder may easily detach from the coating and enter the lungs, increasing inflammatory response. While rose bengal is safely implemented in medicine, we suspect that a laser source at the bedside may be difficult to manage; furthermore, UV light use requires technical expertise and caution. Therefore, after thorough research on the various coatings, we selected the ETT internally coated with silver sulfadiazine in polyurethane. In the in-vitro study, SSD-ETT showed bactericidal properties against *P. aeruginosa*, preventing biofilm formation on the ETT lumen throughout the 72-h experiments. The SSD-ETT was also associated with decreased bacterial colonization of the ETT, ventilator circuit, and lower respiratory tract in sheep mechanically ventilated for 24 h in absence of antibiotic use. No local or systemic adverse

events were encountered. The present in-vitro and sheep studies demonstrated the biological plausibility of using a coated ETT to prevent hospital-acquired respiratory tract infections.

However, our study presents some limitations. First, the in-vitro study assessed colonization of *P. aeruginosa*. No other bacteria were studied. Other bacteria might interact differently with the SSD coating. We chose *P. aeruginosa* because it is the most common biofilm-forming bacterium on medical devices and it is a frequent cause of VAP. Specifically, we used the same strain of *P. aeruginosa* (strain PaO1 containing the GFP-plasmid, pMRP9-1 carbenicillin resistant) used in a recent study to mimic a human biofilm model [25]. In that study Singh et al. showed the temporal sequence of a typical biofilm: adhesion occurs within the first 4 h, and after 24 h those bacteria form microcolonies. At day 3, microcolonies become confluent and cover the entire surface. By day 7, towering pillar and mushroom-shaped biofilms develop.

Second, the animal study was limited to a 24-h period. However, the main goal of this research at this stage was to fabricate effective and safe antibacterial coatings to prevent bacterial colonization of the ETT, rather than decreasing the incidence of VAP.

In a clinical study, we tested the SSD-ETT in patients intubated and mechanically ventilated for up to 24 h. No bacteria were found in the ETT lumen and in the lower respiratory tract of patients in the SSD-ETT group (Berra et al., manuscript submitted [31]). Using a different coating, Rello et al. showed in a prospective, randomized, single-blind, multicenter study that during the first 7 days of intubation coated ETT are associated with delayed colonization on the tube compared with non-coated ETT, and with a lower bacterial burden in tracheal aspirates [13].

Further studies should focus on developing novel antibacterial coatings and should evaluate in the clinical setting whether a decrease in bacterial colonization is associated with favorable clinical endpoints.

Conclusion

The prevention of formation of a bacterial biofilm within ETT is a challenging and expanding field. We fabricated several effective and durable antibacterial coatings for ETT. We propose the use of ETT internally coated with silver sulfadiazine in polyurethane in patients who are intubated and mechanically ventilated.

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