# Assessment of Nanomodified Endotracheal Tubes in a Benchtop Airway Model

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**Abstract:** Ventilator associated pneumonia (VAP) is a serious and costly clinical problem. Patients receiving mechanical ventilation over 24 hours have an increased the risk of contracting VAP, which is associated with high morbidity, mortality and medical costs. VAP is especially difficult to diagnose in children because of non-specific clinical and radiographic signs as well as a lack of sensitive diagnostic methods. Cost effective endotracheal tubes (ETTs) that are resistant to bacterial infection would help to prevent this problem. The objective of this study was twofold, first to develop an *in vitro* airway model to simulate the dynamic conditions of the pediatric airway and secondly to assess *in vitro* bacterial adhesion or biofilm formation on clinically used ETTs and nanomodified (with surface features less than 100 nm in at least one direction) ETTs under these dynamic conditions. In preliminary tests, nanomodified polyvinyl chloride (PVC) ETTs have been shown to be effective at reducing bacterial adhesion. To evaluate the bacterial resistance of these ETTs more effectively, this study designed a bench top airway model to create a similar environment to the natural flow system that ETTs are exposed to *in vivo*.

The airway model designed to test ETTs has two plexiglas chambers representing the oropharynx and the lungs, a tube representing the trachea and finally an intricate pumping system to the oropharynx with bacteria flow and to the lung with simulated compliance and resistance. Endotracheal tubes will be connected to a ventilator and will pass through the oropharynx chamber into the trachea and will be observed under both mechanical ventilation and continuous contamination within the artificial flow system. In no less than three separate trials in the airway chamber, each ETT will be tested for its effectiveness at the reduction of bacterial growth within the airway by sampling from both lung and oropharynx chambers during continuous operation. Special attention will be given to the long-term effects on the ETT by including a study that lasts longer than five days. Both the bacterial proliferation in the two chambers and on the ETT itself will be carefully analyzed. This specialized testing should yield valuable information on the efficacy of nanomodified ETT in airway conditions and will provide further evidence to determine if nanomodified ETTs are a valid solution to VAP.

**Keywords:** Nanotechnology, bacteria, endotracheal tubes, air flow.

## INTRODUCTION

Ventilator associated pneumonia (VAP) is a serious and costly problem. It is one of the most common causes of hospital associated infection in children and adults. This medical condition affects eight to 28% of all patients receiving mechanical ventilation. Depending on the pathogen involved, the patient's underlying condition, and the length of intubation, VAP can have a high mortality rate, ranging between 38% and 76%. *Pseudomonas aeruginosa* (*P. aeruginosa*) is the most common gram negative organism associated with VAP, but multi-drug resistant strains of bacteria are becoming an increasing problem in the treatment of VAP. Two notable strains, *P. aeruginosa* and Methicillin-resistant *Staphylococcus aureus* (MRSA) are of particular concern to clinicians because of their increasing prevalence within hospitals. VAP can also be very costly for both patients and hospitals.

This condition adds an estimated $40,000 dollars to each hospital admission due to the extra intensive care required for treatment [1]. Diagnosis of VAP is especially challenging in pediatrics. Radiographic and clinical criteria for such diagnoses are often unspecific causing delays in targeted treatment and increasing the use of a broad-spectrum of antibiotics. Specifically, tracheal colonization must be differentiated from lower respiratory infection. Cultures taken from patients with suspected pneumonia are often inaccurate, yielding false positives by detecting benign bacterial colonization or false negatives by missing active areas of bacterial infection. Thus, more than half of patients diagnosed with clinical VAP have negative cultures [2]. The diagnosis of VAP in pediatric patients is hindered further by the lack of VAP studies in children and infants.

Endotracheal tubes, one of the main sources of bacterial colonization in the airway, are essential to the process of mechanical ventilation. Like any other device implanted within the body, ETTs interact with an environment that contains not only the airway epithelium, but also potentially harmful microorganisms. Endotracheal tubes present a special concern to clinicians because they are often colonized by oropharyngeal bacteria during long-term mechanical ventilation. These tubes provide a conduit from the outside environment to the more sterile area of the lungs

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by impairing some of the body's natural defenses like coughing and muscociliary motion of the trachea.

The mechanical stress placed on ETTs in the patient can cause even more problems. Opportunistic adherence of bacteria to the airway is one such issue. It is caused by injury to the epithelial cells of the trachea that can result from the movement of the tube in the airway or even from the suction of secretions during nursing care. An additional problem caused by ETTs occurs when these tubes cause chronic airway inflammation. Inflammation releases proteases that digest fibronectin. Fibronectin bound to the buccal epithelial surface has been shown to inhibit binding of certain gram negative bacteria. Fibronectin is an oposonin, mediating phagocytosis by macrophages. Recently, the gel phase of mucus in the trachea has been shown to have high levels of fibronectin and this glycoprotein is thought to aid in the mechanical removal of bacteria [3]. Endotracheal tubes can also increase airway secretions creating a substrate for *P. aeruginosa*, a bacteria that binds to receptor sites on the mucus [4].

One of the key challenges towards the inhibition of bacterial growth on ETTs occurs when certain bacteria exude an exopolysaccharide, which adheres bacteria together. Bacteria in this type of extracellular matrix (known as a biofilm) are especially resistant to both antibiotics and the immune system of the patient. Ventilation through these infected tubes, or even condensation of humidified air can break off pieces of this biofilm bringing bacteria deeper into the lungs and spurring growth on other areas of the tube [5].

Increased secretions as well as biofilm formation on ETTs themselves have been shown to change the cross sectional area of ETTs. Recent studies have shown significant changes in tube resistances when obstructions reach certain critical values [6]. This can interfere with oxygen delivery and cause serious strain on the patient.

In the past, efforts to reduce VAP have concentrated on decreasing bacterial contamination during intubation by modifying medical procedures. It has become increasingly apparent that the elimination of bacteria also depends on the reduction of bacterial growth on the ETTs.

Cost effective ETTs that are resistant to bacterial infection provide an essential tool to prevent VAP. Nanomodified devices used within the body mimic their natural surroundings and subsequently often lack detrimental immune responses to the foreign object. Research shows a connection between nanophase surfaces (that is, surfaces with features less than 100 nm in at least one direction) and increased bacterial metabolism suggesting the topography of nanostructures influence the biological processes of bacteria [7]. Addition of nanomaterials to these bacteria modifying surfaces could enhance their anti-microbial properties. Preliminary research has uncovered a number of materials that could produce an effective antimicrobial ETT. These materials display antibacterial properties using a number of different methodologies to interfere with bacterial growth and adhesion. Many of these nanomaterials have also been shown to increase fibronectin adsorption thus inhibiting bacterial growth.

However, these preliminary studies were performed under static conditions and do not take into account the dynamic forces that occur in the human airway. Our model seeks to characterize the antimicrobial properties of nanomodified ETTs within a continuously contaminated airway model. This study hypothesizes that airflow and the impact of continuous bacterial contamination on ETTs are essential for the determination of the bacterial resistance of these tubes in the pediatric airway.

## METHODS

The first type of antibacterial ETT tested in our system was polyvinyl chloride (PVC) ETT with nano-roughened surfaces. These tubes were created using lipases from the bacteria *Candida cilindracea* and *Rhizopus arrhisus* (Sigma Aldrich) which enzymatically degrade the PVC material. The procedure included exposing the PVC tubing to a 0.1% mass solution of either *C. cilindracea* or *R. arrhisus* lipase dissolved in a potassium phosphate buffer at 37° C. The samples were then gently agitated for 24 hours at which point they were washed with distilled water and exposed to fresh enzyme solution. Thus, the tubes remained in contact with the lipases for a total of 48 hours. The activity of the *R. arrhisus* used in the experiment was 10.5 U/g and that of *C. cilindracea* was 7.29 U/g/cm$^2$ where one unit was defined as the amount of enzyme that catalyzed the release of 1 μmol of oleic acid per minute at a pH 7.4 and 40°C [8] (Fig. **1**).

Each of the samples was coated with a gold palladium mixture to increase conductivity. Then, the tubes were analyzed using an atomic force microscope (AFM). Endotracheal tubes placed in the system were only modified on the inner surface.

Static studies were performed to analyze two bacteria commonly found in VAP, *Pseudomonas aeruginosa* (ATCC #25668) and *Staphylococcus aureus* (ATCC #25923). These two bacterial strains were inoculated into trypticase soy broth (TSB) media and at 4, 12, 24, and 72 hour time points for TSB media. The bacteria found on these samples were stained with crystal violet and quantified using optical density.

The ultimate test of the effectiveness of nanomodified ETTs under the conditions present within the airway resulted in the construction of a bench top model. Hartmann *et al*. (1999) created a continuously contaminated airway system to test the effectiveness of the silver coated endotracheal tubes (SCET) [9]. The airway model for this study was based upon this general design. Fig. (**2**) contains an illustration of the Hartmann model while Fig. (**3**) depicts the design changes implemented for this study.

The model in Fig. (**2**) contains two polymethylmethacrylate (Plexiglas) chambers that simulate the structures found in the airway. The first chamber, or oropharynx-larynx box, simulates the conditions found in the oropharynx- larynx and the second chamber, or lung box, simulates the compliance and resistance found within the lung. A Plexiglas tube, representing the trachea, connects the two boxes. Cuffed nanomodified ETTs run through the oropharynx chamber, where they are exposed to continuous bacterial media, into the trachea, where cuff pressure conditions dominate.

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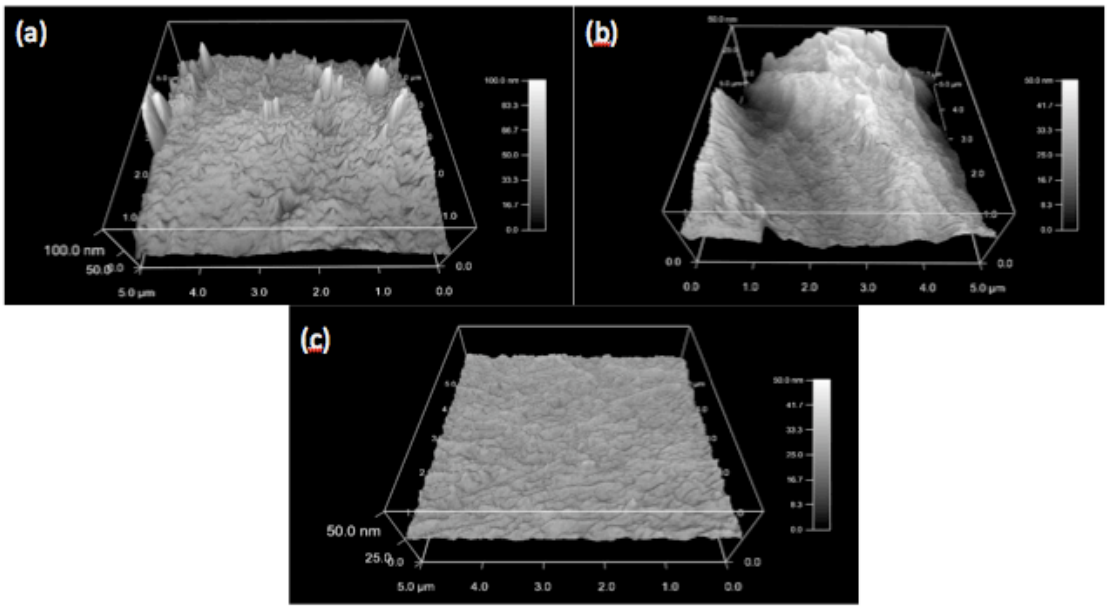


**Fig. (1).** AFM micrographs of PVC samples treated with (**a**) 0.1% *R. arrhisus* solution and (**b**) 0.1% *C. cilindracea* solution compared to (**c**) untreated PVC samples after 48 hours.

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The oropharynx-larynx box constructed from Plexiglas was filled with 100 mL of bacterial media. Bacterial media was continuously added to the system using a peristaltic pump (Curlin 4000 CMS). This additional media was either drained down the trachea or out of an overflow valve on the side of the chamber. Endotracheal tubes were submerged in this continually circulating bacterial media, not only providing important information on the effects of dynamic conditions on ETTs, but also more accurately simulating the conditions of resistance in the pediatric trachea. The length and width of the oropharynx-larynx chamber are based upon the length of the oropharynx and larynx of an 18 month old child.

It is important to note that the system contains a method to agitate the chamber before samples were taken using an additional Curlin pump. This allowed us to take samples from the system that are homogeneous and that accurately represent the total bacteria content in the oropharynx. Agitation also will prevent any sedimentation of cells within the chamber. This chamber constitutes the first step towards the simulation of the conditions found in the body during mechanical ventilation, because it places the mechanically ventilated ETT in direct contact with bacteria and media.

The second part of our model is a Plexiglas tube that represents the trachea. This tube simulates the pressures on the ETTs cuff *in vivo*. The end of this tube was exposed to airflow from the ETTs. The diameter of this tube was calculated based on an average of the thoracic inlet tracheal size, aortic arch tracheal size and supracarinal tracheal size (Equations 1, 2, and 3) of an 18 month old child with a height (H) of 80 centimeters.

$$\text{Thoracic inlet} = (0.478 * H) - (0.00446 * H^2) - (0.0000147 * H^3) - 9.80 \pm 1.94 \quad (1)$$

$$\text{Aortic arch} = (0.358 * H) - (0.00324 * H^2) - (0.0000108 * H^3) - 5.88 \pm 1.68 \quad (2)$$

$$\text{Supracarinal} = (0.388 * H) - (0.00346 * H^2) - (0.0000115 * H^3) - 7.02 \pm 1.69 \quad (3)$$

The lung is the last organ modeled within the system. It is represented by a dual chambered Plexiglas box. This simple model simulates both the resistance and compliance of the lung. These two factors are the most important mechanical stresses on the airway system and are important for accuracy using the ventilated model. The total volume of the two chambers was 400 mL and they were filled with 236 mL of media. The geometry of the lung chamber was based upon the compliance of the lung. The compliance in an 80 cm, 18 month old child was determined to be 34 mL/cm$H_20$ by equation 4.

$$C_{lung}\ (\text{ml/cm } H_2O) = 0.0017 * \text{length}^{2.26} \quad (4)$$

The resistance of the lungs was determined to be 40 cm$H_2O$/liters/second and is simulated by a Plexiglas plate with 64 boreholes with a diameter of 6.4 millimeters each using equation 5 where $\Delta p/Q$ is the resistance (dynes/ cm), $\eta$ is the viscosity, L is the length of the sieve tube (cm), R is the radius of the sieve tube (cm), r is the sieve pore radius (cm), l is the sieve plate thickness (cm), and N is the total number of pores.

$$\Delta p = \frac{8}{\pi} \frac{\eta Q}{R^4} \left[ L + \frac{l}{N} \left( \frac{R}{r} \right)^4 \right] \quad (5)$$

The model was connected to an Infant Star 950 ventilator. It was tested at an inspiratory flow rate of 8.5

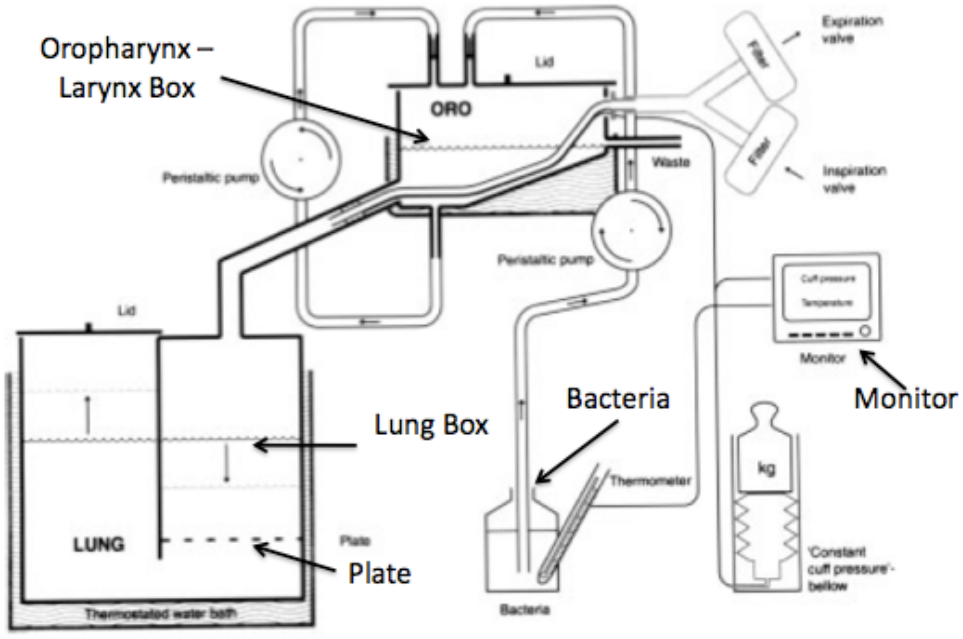


**Fig. (2).** Diagram of model airway system.

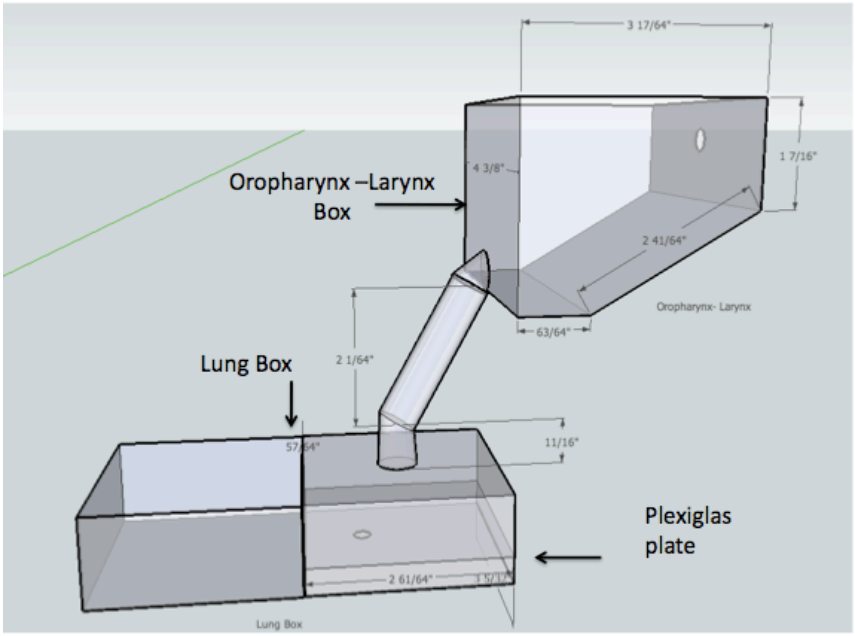


**Fig. (3).** Diagram of Plexiglas pediatric model.

mL/sec. In addition the positive end-expiratory pressure (PEEP) was set at 5 $cmH_2O$. This allowed for a peak airway pressure of about 11.7 $cmH_2O$ at a tidal volume of 100 mL [10, 11]. The fraction of inspired oxygen ($FiO_2$) was maintained at a value of 1 for this trial, to limit flow variability.

Other features of the system that are essential to the experiment are the many controls that make the data reproducible and lessen confounding influences: such as lids for the exposed areas of the lung and the oropharynx chambers to lower contact with the surrounding environment as well as lessen the chance for potential outside contamination. To better reproduce the conditions of the body, both the oropharynx-larynx chamber and the lung chamber were immersed in water baths keeping these chambers at a constant 37° C. Humidified air was also used in the system in order to better replicate clinical conditions. This is an important feature of the airway model because humidified airflow through contaminated ETTs has been shown to increase bacterial transport to the lungs. The mechanism of this transport is thought to be the removal of bacteria from biofilms formed on the ETTs by the condensation of humidified air [6].

Cuff pressure and temperature of the bacterial media were measured by a portable monitor (Datascope Passport 2), while humidity was recorded in the lung box and the

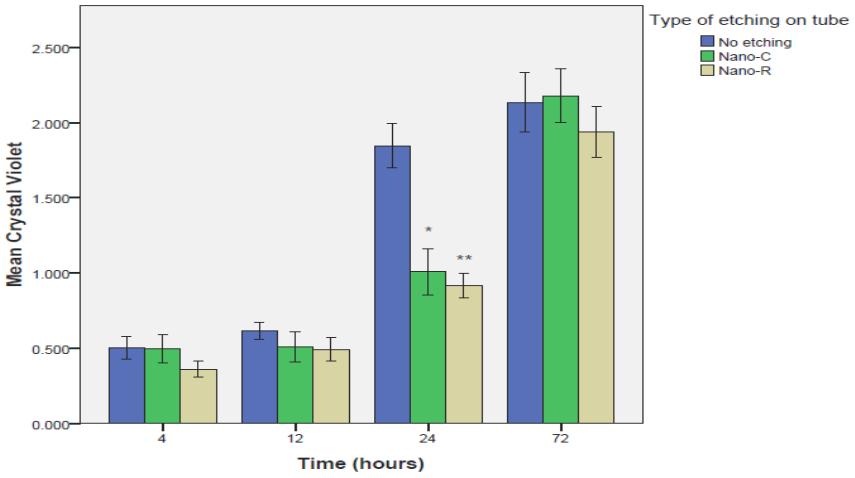


**Fig. (4).** *Pseudomonas aeruginosa* in TSB, N=3: Error bars +/- 1 SE, **p* < 0.05 ***p*< 0.05 compared to controls.

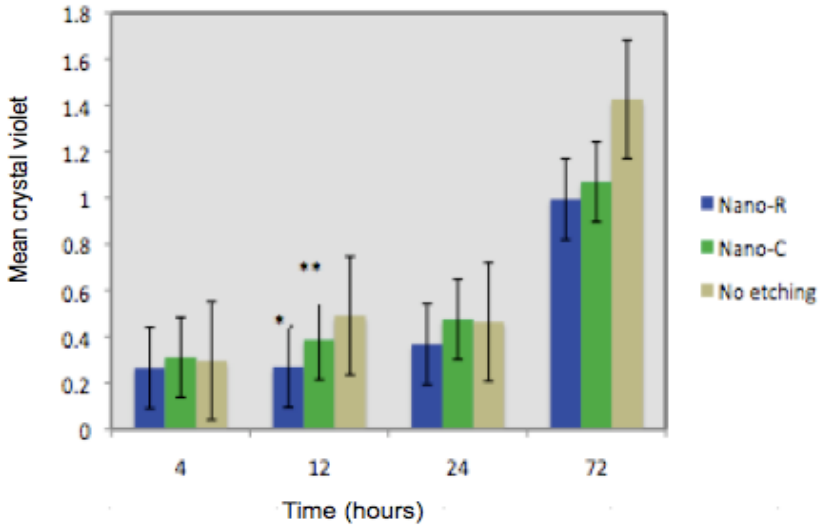


**Fig. (5).** *Staphylococcus aureus* in TSB, N=3; Error bars +/- 1 SE, **p* < 0.05 ***p*< 0.05 compared to controls.

oropharynx using two Varisala HMT337 sensors. A reduced TSB media was used within the model.

Finally to quantify the number of bacteria within the system, 2 mL samples of the fluid in the oropharynx chamber and the lung chamber were cultured at regular intervals. The first experiment used intervals of 4, 12, 24, and 48 hours to correlate with static studies. The system remained running during sample collection. These samples were diluted, plated, and incubated for 48 hours. The number of colonies that formed helped to determine the effectiveness of the ETT. The ETT were massed before and after each experiment and biofilms on each section of the tube analyzed using a crystal violet stain.

## RESULTS

This first treatment of PVC tubes cut and submersed in either lipase solution (*C. cilindracea* or *R. arrhisus*) showed visible nanofeatures on the ETT tube surface. Whereas untreated PVC did not have nanofeatures.

The average root mean square roughness was 12.5 nanometers for *R. arrhisus* and 14.7 nanometers for *C. cilindracea* compared to a value of 2.2 nanometers for the untreated PVC tubes. Surface energy analysis for the nanomodified PVC was also performed giving a water contact angle of 84.4˚ for unmodified PVC, an angle of 66.2˚ for *C. cilindracea* and 68.9˚ for *R. arrhisus.*

Results for the static studies performed within the TSB can been seen in Figs. (**4**, **5**). Statistically significant differences between the untreated tubes and the nanomodified PVC tubes were seen at all time points for *S. aureus* and at the 24 hour time point for *P. aeruginosa.* The mechanism for this reduction on the nanomodifed tubes is unclear but most importantly is associated with the reduced adhesion of the bacteria to the surface of the tube possibly due to altered initial protein interactions.

## DISCUSSION

Past evaluations of pediatric ETT effectiveness have concentrated on either static contamination or dynamic airway simulations. This study aimed to simulate these important attributes of the human airway and to use this knowledge to better quantify the antimicrobial properties of

ETTs. A number of unique aspects included within this study are the introduction of humidified air to the system, the media used for the samples, and even the redefined size and shape of the two chambers that simulate the lung and oropharynx. These parameters not only separated this study from its predecessors, but also yielded valuable insights into bacterial colonization and proliferation on ETTs. Moreover, the studies on the anti-microbial nature of the nanomodified ETT have shown significant reductions in bacteria growth at the 12 and 24 hour time points. These results could be the effect of increased protein interactions with the more hydrophilic nanomodified surfaces. Future work will further investigate the nature of this initial decrease in bacterial adhesion and will focus on the influence of dynamic conditions on nanomodified ETT in order to better understand their effectiveness *in vivo*.

## CONCLUSIONS

These *in vitro* studies have shown that chemical etching with bacterial lipases can create nano-rough surface features on PVC in an inexpensive but effective manner. Nanomodified tubes also have been shown to inhibit *P. aeruginosa* growth in tryptic soy broth media. Additionally, *S. aureus* growth was significantly reduced on nanomodifed ETTs at all time points compared to untreated ETT in TBS. These results suggested that these nanomodified tubes could provide clinicians with an effective tool to combat hospital acquired infections like VAP and should be studied in greater depth.

## ACKNOWLEDGEMENTS

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## ABBREVIATIONS

ETT = Endotracheal tube

VAP = Ventilator Associated Pneumonia

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