Evaluating Bacterial Colonization of Nanomodified Endotracheal Tubes in a Bench Top Airway Model

Mary C. Machado[1], Keiko M. Tarquinio[2], and Thomas J. Webster[3]
[1]School of Engineering, Brown University, Providence RI, 02912; [2]Division of Pediatric Critical Care Medicine, Rhode Island Hospital, Providence RI, 02912; [3]School of Engineering and Department of Orthopaedics, Brown University, Providence RI, 02912

Statement of Purpose: Ventilator associated pneumonia (VAP) is a serious and costly clinical problem. Specifically, receiving mechanical ventilation over a 24 hour time period increases the risk of VAP and is associated with high morbidity, mortality and medical costs. Diagnosis is especially difficult in children because of non-specific clinical signs and diagnostic methods that are not applicable to these patients because of their size. Cost effective endotracheal tubes (ETTs) that are resistant to bacterial infection would be essential tools in the prevention of VAP. The objective of this study was twofold, first to develop strategies to decrease bacterial adhesion on ETTs and secondly to develop better methods to assess *in vitro* bacterial adhesion and biofilm formation on ETTs using a bench top experimental model and computer simulation of flow.

Methods: Nanomodified tubes were created using a chemical etching process where the tubes were enzymatically degraded by a 0.1% mass solution of one of two bacterial lipases, *Candida cilindracea* (Nano-C) or *Rhisopusarrhisus* (Nano-R). These tubes were then evaluated in two different ways, static studies and a bench top airway model that simulated the dynamic airway conditions found *in vivo*. Static studies were performed on *S. aureus* (ATCC #25923). *S. aureus* was inoculated into trypticase soy broth (TSB) media.

Polyvinyl chloride (PVC) was then immersed into the inoculated media and into a control containing media without bacteria. Bacterial growth on the surface of the PVC was assessed at 4, 12, 24, and 72 hour time points. The bacteria found on these samples were quantified using optical density after crystal violet staining.

Additionally, this study sought to evaluate the bacterial resistance of these ETTs more comprehensively by testing these tubes in a bench top airway model that simulated mechanical ventilation and continuous contamination which ETTs are exposed to *in vivo*. The airway model designed for this purpose contained two polymethylmethacrylate chambers. The top represented the oropharynx and bottom dual chambered box, the lungs. The boxes were connected by a tube representing the trachea. This pediatric sized model was based upon the work of Guttman et al. (1999). ETTs were loaded into the system and samples were taken from the oropharynx and lung boxes at 0,12, and 24 hours. Bacterial concentration in both chambers and on the ETT was again characterized using a crystal violet assay.

Results: Results showed that nanomodified PVC ETTs were effective at reducing bacterial colonization in static studies (Figure 1). *S. aureus* was reduced on the nanomodified ETTs at all time points in the TSB media.

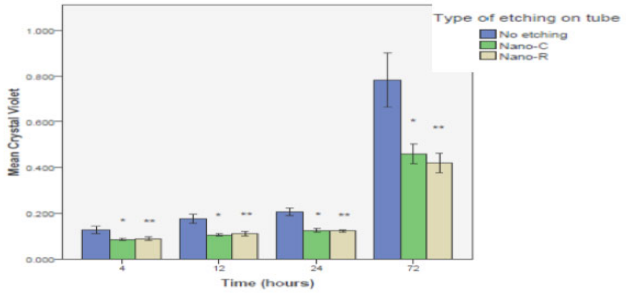

Figure 1. Static *S. Aureus* mean crystal violet staining on nano-structured PVC ETT, N=3; Error bars +/- 1 SE, $*p < 0.05$;$**p < 0.05$ compared to controls at same time points

Results of the dynamic lung system tests on untreated tubes can be seen in Figure 2. Mean crystal violet (optical density) is plotted over the length of the tube. Interestingly, results demonstrated uneven bacterial growth over the tube, which was correlated to air flow models and potentially turbulence. Dynamic results on nanomodified PVC will be presented.

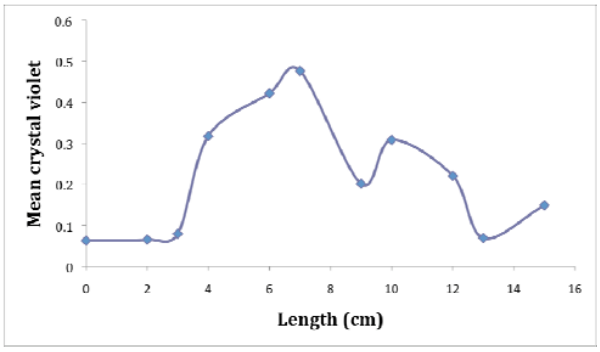

Figure 2. Dynamic lung system results for untreated tubes, N=3 (x-axis length= length along endotracheal tube, mean crystal violet= staining of *S. aureus*)

Conclusions: Chemical etching techniques can create nano-rough surface features on PVC that inhibit *S. aureus* growth in static studies. In addition, dynamic lung conditions have an effect on both the concentration and location of bacterial growth on the ETT. Fluid effects of the bench top model will be further investigated using a computational model, to explain the unique patterns of bacterial growth seen within the experiments.

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Reference: Hartmann, M.; Guttmann, J.; Muller, B.; Hallmann, T.; Geiger, K.; *Technology and Health Care*, **1999**, 359-70.