Comparison of Quantification Methods Illustrates Reduced *Pseudomonas Aeruginosa* Activity on Nanorough Polyvinyl Chloride

Keiko M. Tarquinio[1], Justin T. Seil[2], Nathan M. Rubien[3], Thomas J. Webster[2]
[1]Pediatric Critical Care Medicine, Hasbro Children's Hospital, Rhode Island Hospital, [2]School of Engineering, Brown University, [3]Rhode Island Hospital, Lifespan, Providence, RI, 02917

Introduction: Ventilator associated penumonia (VAP) is one of the most common nosocomial infections among intensive care unit patients, which prolongs mechanical ventilation and hospitalization, resulting in significant morbidity, mortality and medical costs. The process of introducing an endotracheal tube (ETT) into the trachea is clearly responsible for the entrance of pathogens, likely causing the development of biofilms on ETT. Despite advanced approaches to VAP in adult sized ETTs (Hi-Lo Evac® [Nellcor Puritan Bennett, Boulder, CO: ETT with secondary suction port attachment] or Agento® [C. R. Bard Inc., Convington, GA: silver coated ETT]) to overcome this problem, the cost effectiveness and utility in the pediatric population have not been well studied. Applying nanotechnology to modify ETT materials without such coatings is an attractive alternative. This study compared the growth of *Pseudomonas aeruginosa*, one of the most common pathogens in late onset VAP, on conventional ETTs and on nano-modified ETTs while comparing different methods of biofilm quantification.

Methods: Commercially available polyvinyl chloride Seridan® 6.0mm ID, uncuffed ETTs (Hudson RIC, Temecula, CA) were cut and soaked in *Rhizopus arrhizus* lipase (Sigma-Aldrich, St. Louis, MO) for over 48 hours to create a nanorough (Nano-R) topography. These samples were analyzed for surface topography and surface chemistry using scanning electron microscopy (SEM), X-ray photoelectron spectroscopy and atomic force microscopy (AFM). After sterilizing ETTs, 150 µl of *Pseudomonas aeruginosa* (ATCC 25668, American Type Culture Collection, Manassas, VA) diluted 1:30 was inoculated on Nano-R and untreated ETTs, and incubated at 37 °C with 5 % carbon dioxide for 24 hours. The media and non-adherent bacteria were carefully rinsed from sample surfaces with phosphate buffered saline. Each sample was then placed into a scintillation vial containing 2ml of tryptic soy broth (TSB).

Biofilm removal: Four different processes were compared to test the removal of bacteria from ETTs for quantification.

Method 1: Vortexing for 1 min
The vial was held in a vertical position and vortexed for 1 min at 3000 rpm.

Method 2: Ultrasonication for 10 min
The vial was placed in a tabletop ultrasonic cleaner (B2500A, VWR International, Batavia, IL) and sonicated for 10 min at the highest setting. The output of the ultrasonic cleaner was 135 W at a frequency of 42 kHz.

Method 3: Vortexing and Ultrasonication
All samples were vortexted for 1 min first, then sonicated for 10 min subsequently.

Method 4: Vortexing with Tween® 80 (Sigma-Aldrich, St Louis, MO)
The sample was vortexed for 1 min at 3000 rpm in polysorbate 80 (commercially available surfactant or Tween® 80) with TSB (5% by volume).

The supernatant media derived from the 4 methods above were diluted and plated on tryptic soy agar plates for viable cell counts. Following biofilm removal, samples were also fluorescently labeled with a Live/Dead® stain (*Bac*Light, Invitrogen, Carlsbad, CA) to observe any remaining bacteria on the ETTs surface. These samples were imaged on a fluorescence microscope (Leica DM5500B, Leica Microsystems, Wetzlar, Germany). All experiments were run in triplicate and analyzed for significance using the student's *t*-test.

Results: The presence of a nanorough surface was confirmed with SEM and AFM, and surface chemistry was confirmed unchanged compared to untreated ETTs (chlorine and carbon peaks) using spectroscopy. For all methods of bacteria removal, the bacterial yield was greater for conventional ETTs compared to Nano-R ETTs, illustrating reduced bacteria on the Nano-R ETTs. Results further indicated that the ultrasound method didn't effectively remove biofilm from the ETT surface (Fig. 1). Live/dead staining showed minimal residual bacteria, confirming that methods 1 & 4 removed sufficient bacteria from the surface.

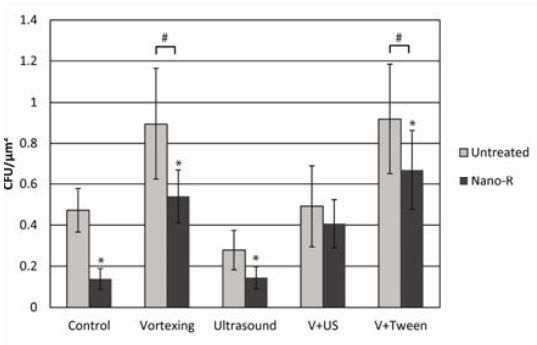

Fig 1. Colony forming units (CFU) of *Pseudomonas aeruginosa*. Data = mean +/- SEM; N = 3; *$p < 0.05$ compared to untreated samples under same conditions, $^{\#}p < 0.05$ for both untreated and Nano-R compared to control.

Conclusions: Vortexing with and without 5 % Tween 80® adequately removed *Pseudomonas aeruginosa* biofilms from conventional and Nano-R PVC ETTs. PVC nanoroughness reduced either the adhesion or proliferation of cultured bacteria. Further mechanisms of bacterial adhesion to PVC surfaces need to be investigated in future studies.

Acknowledgements: The authors would like to thank STAC for funding.