Reduced Adhesion of *Staphylococcus aureus* to ZnO/PVC Nanocomposites

Benjamin M. Geilich, Thomas J. Webster.
Northeastern University, Boston MA

Statement of Purpose: Bacterial infection is one of the most common complications associated with implanted medical devices. Contamination frequently occurs from a very small number of microorganisms, and at least 50% of all nosocomial infections are device related[1, 2]. Ventilator-associated pneumonia (VAP) is the most common nosocomial infection among patients requiring mechanical ventilation. This infection carries up to a 30% mortality rate, and increases medical costs by an average of over $40,000/incidence[3]. The material surfaces of medical implants/devices are especially prone to bacterial infections. This is because they provide the bacteria with a site to form biofilms on, and thus increase their proliferation and resistance to antibiotic treatment. One of the most common bacteria associated with endotracheal tube infection and VAP is *Staphylococcus aureus*. With the emergence (and increasing prevalence) of strains of *S. aureus* that are especially resistant to antibiotic treatment (MRSA), there is an urgent clinical need to develop new ways of preventing infection without resorting to antibiotics. In this study, the authors' goal was to inexpensively reduce *S. aureus* adhesion to PVC taken from a conventional endotracheal tube by embedding the polymer with zinc oxide (ZnO) nanoparticles.

Materials and Methods: To make the ZnO/PVC nanocomposite material, PVC taken from a commercial Sheridan® 6.0mm ID, uncuffed endotracheal tube (Hudson RIC, Temecula, CA) was first melted using tetrahydrofuran (THF, Mallinckrodt Chemicals, St. Louis, MO) at a ratio of 1g PVC : 4mL THF. Once the PVC was completely melted, 20nm diameter ZnO nanoparticles (mkNANO, Williamsville, NY) were added to the mixture in weight percentages of 0%, 10%, 20% and 30%. The PVC/ZnO mixture was left to stir for 1 hour, and then sonicated for 15 minutes to ensure an even distribution of the nanoparticles. The mixture was then pipetted onto 12mm circular glass coverslips and allowed to dry in the fume hood overnight. For bacteria studies, *Staphylococcus aureus* (strain ATTC#25923) was used. The ZnO/PVC nanocomposites were removed from the glass coverslips and 4 samples of each weight percentage were placed into separate wells of a 24-well plate. 1mL of a 10^6 CFU/mL solution of *S. aureus* in prepared Simulated Body Fluid (SBF) was inoculated into each well and allowed to incubate at 37ºC for 24 hours to allow for biofilm formation. The bacteria solution was then discarded, and the samples were washed three times with phosphate buffer solution (PBS, Fischer Scientific, Fair Lawn, NJ) to ensure removal of all un-adhered bacteria. Samples were then placed in 1.5mL microcentrifuge tubes (Fischer Scientific, Fair Lawn, NJ) with 1mL of PBS and vortexed for 15 minutes. This solution was serially diluted in PBS over a 3-log range, and 200µL of the final solution was spread onto agar (Fischer Scientific, Fair Lawn, NJ) plates and allowed to incubate at 37ºC for 24 hours. Finally, the number of *S. aureus* colonies that formed on each plate was recorded.

Results and Discussion: Figure 1 shows the anti-biofilm activity of the ZnO/PVC nanocomposites. Bacterial adherence was drastically decreased by the addition of just 10% ZnO nanoparticles (71% reduction from the control), and further decreased by the addition of more nanoparticles (82%, 87% reduction from the control for the 20%, 30% ZnO concentrations). Figure 2 shows a confirmation of these results- 5 20µL dots of solution from each sample were placed on the same agar plate and allowed to incubate for 24 hours. The authors are currently exploring the mechanism behind this reduction.

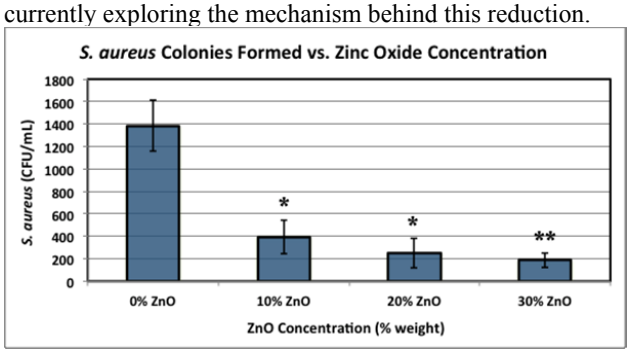

Figure 1: Anti-biofilm activity of different weight percentages of ZnO-embedded PVC against *S. aureus* adherence after 24 hours. Data represents mean ± SD; n=8. Fewer bacteria than control at confidence *p<0.001, **p<0.0001.

Figure 2: Agar plate with 5 20µL drops of bacteria solution taken from each sample (0% bottom, 10% right, 20% top, 30% left).

Conclusions: Our results have important implications. We have shown that embedding conventional PVC used to manufacture endotracheal tubes with ZnO nanoparticles drastically lessens the adherence of *S. aureus*. It is envisioned that this simple and inexpensive approach could lead to a reduction in nosocomial infections, longer medical device lifetimes, and decreased antibiotic usage and reliance. All of these events could lead to decreased antibiotic resistance and patient infection rate in the clinical setting. The authors intend to further characterize the surface features of the composites, with the goal of gaining insight into the mechanisms behind the biofilm reduction. Testing efficacy against common pathogens other than *S. aureus* is also planned.

References: [1] Hall-Stoodley L. *Nat Rev Micro.*, 2004; 95-108. [2] Burke JP, *N Engl J Med.* 2003;651-659. [3] Seil JT. *J Biomed Mater Res B Appl. Biomater.,* 2011;1-7.