An *in vitro* Operating-Room Model of Bacterial Contamination

Wenhan Zhao,[1] Matthew Libera,[1*] Meredith Prysak,[2] Jordan Katz,[2+] and Lauren De Stefano[2]
[1] Dept. of Chemical Engineering and Materials Science
Stevens Institute of Technology, Hoboken, NJ. USA
[2] Orthobond Corporation
Princeton, NJ. USA

Statement of Purpose: Infection associated with tissue-contacting biomedical devices is a compelling clinical problem initiated by the microbial colonization of the device surface. One source of contaminating bacteria is the surgical operating room (OR) itself, where bacteria can sediment into the surgical site or onto a medical device. Here we describe a system that can spray well-defined small quantities of aerosolized bacteria and illustrate an in vitro model of device contamination in the OR where sedimentation rates are ~10^3-10^4 CFU/m^2-h.

Methods: A bacterial aerosol apparatus was developed to mimic the OR contamination (Figure 1 left). Three unmodified Ti coupon surfaces and three Ti coupon surfaces modified with a quaternary ammonium compound (QAC) were sprayed with Methicillin Sensitive Staphylococcus aureus (MSSA, ATCC 29213) or Staphylococcus epidermidis (*S. epidermidis*, ATCC 35984). The optical density of each bacterial suspension in PBS prior to spraying was varied from OD_{600} = 0.002A to 0.0005A with a target volume of 40 ml. A single shot (100 ms) was sprayed onto one side of each coupon. After a 30 min drying period, each coupon was immersed in 2 ml PBS and sonicated for 10 min. Quantification of the net contamination was determined by the sum the number of loosely bound colonies recovered by sonicating in PBS and the number of well-adhered colonies grown directly on the sonicated coupons after covering with an agar overlay. The bacterial distribution was estimated by spraying directly onto TSA plates followed by culture. The reproducibility was assessed by spraying MSSA (0.0005A) on the unmodified control coupons (n=3) on three different days.

Results: Figure 1 (right) shows the spray pattern on TSA plates. There is a relatively uniform distribution of bacteria with a slightly decreasing density moving radially outward from the plate center. The average sprayed density can be controlled using different concentrations of bacterial suspension. Increasing the OD_{600} from 0.0002 A to 0.0005 A increases the average number of sprayed was by a factor of 2.6. For *S. epidermidis*, increasing the OD_{600} from 0.0002A to 0.0004 A, increased in bacterial density by 1.9x.

When using this OR contamination system to spray MSSA on unmodified Ti coupons, over 70% of sprayed bacteria can be recovered either as loosely bound or strongly adhered. Compared with these control samples, QAC-treated Ti coupons have a 99% bacterial reduction rates for both MSSA and *S. epider*midis with statistically significant differences ($p<0.05$) (Figure 2).

Conclusion: A system has been developed to mimic the contamination of a tissue-contacting biomedical device due to bacteria sedimenting from the atmosphere within the OR. In contrast to many models where surfaces are challenged with unrealistically large concentrations of bacteria, this system can reproducibly spray small quantities (~10^2 CFU) onto a test surface. Despite this low level of contamination, statistically significant effects of antimicrobial surface treatments can be demonstrated.

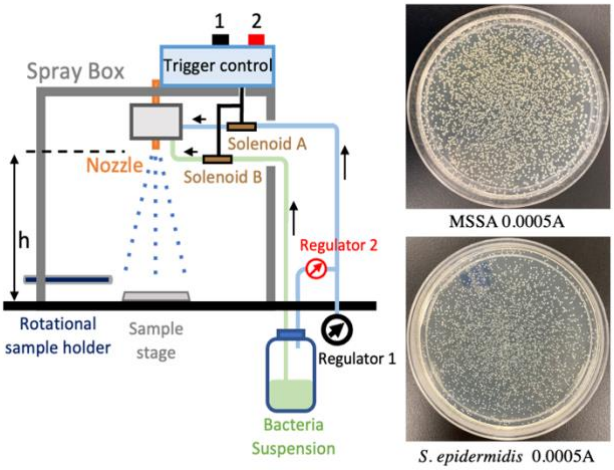

Fig. 1: Schematic description of the bench-top aerosolizing system. The pattern of MSSA and *S. epidermidis* sprayed directly onto TSA plates(right).

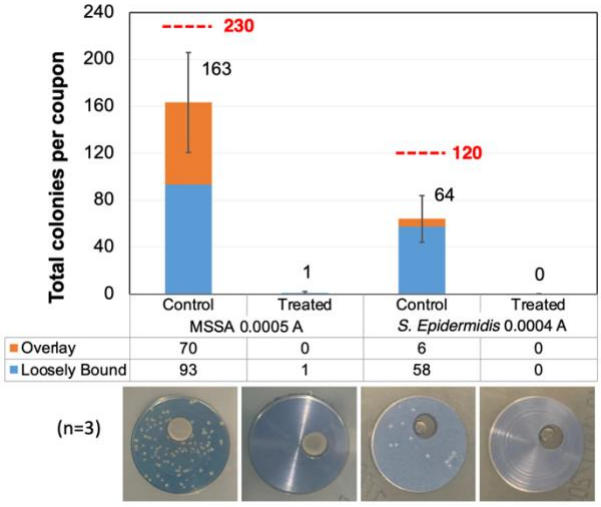

Fig 2: An *in vitro* OR contamination model demonstrates the effectiveness of a Ti surface treated with QACs against MSSA and *S. epidermidis.* The data points/error bars correspond to the average/standard deviation for n=3.