Aerosolized Bacteria Model of Operating Room Contamination of Orthopedic Implants

Amanda R Watkins[1], Oliva Swiber[1], Nicole Gill[2], Scott Korte[1], Gaby Campos[3], Gabriel Costa[3], Nicholas Focarazzo[3], Jennifer Gonzales-Pasion[3], David Davies[2], Lauren DeStefano[4], Jordan Katz[4], Claudia Marques[2], M. Libera[3], Thomas P Schaer[1]
[1]University of Pennsylvania, Philadelphia, PA, [2]Binghamton University, Vestal, NY, [3]Stevens Institute of Technology, Hoboken, NJ, [4]Orthobond Corporation, Monmouth Junction, NJ
watkinsa@vet.upenn.edu

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INTRODUCTION: Contamination of medical devices and implants is a constant concern to surgeons and results in substantial morbidity and mortality for patients. Aerosolized bacteria within the operating room, originating from many sources including air-handling systems, textile materials, and coughing/sneezing by OR staff, are an increasingly recognized source of implant contamination (1). Current *in vivo* models assessing peri-implant infection rely on inoculating the surgical site with an aqueous solution of planktonic microbes at a relatively high concentration (10^6 CFU/mL) or by placing a mature biofilm into the surgical site (2). Neither of these methodologies is a particularly faithful model of implant contamination via sedimentation of airborne particles. An aerosolization system that allows for reproducible delivery of coagulase-positive and negative staphylococcal strains onto a surface using short bursts of pressurized gas has been described (3, 4). This system allows for the inoculation with densities of bacteria as low as of 10^2 CFU/cm^2. This device was designed to be portable and self-contained, allowing us to work with pathogenic strains such as MRSA. It has been shown *in vitro* that this system results in contaminated implants with a quantifiable level of bacterial burden and that implants with anti-microbial functionalized surfaces result in lower bacterial burden than unmodified implants. This tightly controlled apparatus for delivering aerosolized bacteria represents an excellent tool for testing various implant surface modifications using *in vivo* models, which better represent the contamination via sedimentation that occurs in the operating room. As the first step towards this goal, we show here the results of utilizing the spray apparatus to contaminate unmodified Titanium sputter-coated polyetheretherketone implants with a range of inoculum concentrations in a rat model of peri-implant infection.

METHODS: **In vitro**: *S. aureus* (ATCC 25923) was cultured overnight in tryptic soy broth at 37°C with shaking at 235 rpm. 15mL of overnight suspension was centrifuged at 1200×g for 10 minutes and the supernatant was decanted. The bacterial pellet was resuspended in 10 mL of sterile 1X PBS (pH 7.4) via vortexing, then diluted tenfold prior to measurement of optical density (OD) via spectrophotometry at 600nm. Bacteria were subsequently diluted in 1X PBS to achieve the desired dilutions, with a final suspension volume of 20mL. In a laminar flow hood, 8-hole RatFix plates (RISystem) were positioned centrally on 10 cm tryptic soy agar plates using sterile hemostats. The implants were inoculated with a 100 msec aerosol burst of bacterial suspension at 10^2, 10^3, and 10^4 CFU/mL strengths and allowed to dry uncovered at room temperature for 30 minutes. Post-inoculation, plates were transferred into 1 mL of 1X PBS supplemented with 0.3% Tween-20 and subjected to sonication at 40 kHz and 37°C for 10 minutes in a Branson 2800-MH Ultrasonic Cleaner. Recovered colonies were serially diluted to achieve a final dilution of 10^{-6}. From each dilution, 1mL of suspension was seeded onto Aerobic Count Petrifilm (3M) and incubated at 37°C. After 48 hours, colonies were counted and growth expressed as CFU/cm^2. **In vivo**: 8-hole PEEK RatFix plates (RISystem) were prepared as described above with inoculum concentrations of 10^2, 10^3, and 10^4 CFU/mL and allowed to dry. With IACUC approval, male Sprague-Dawley rats were anesthetized, and the lateral femur was accessed via a craniolateral incision between the vastus lateralis and the biceps femoris. Once the plates had dried, the 8-hole RatFix plate was secured with four 0.7mm titanium screws to the lateral aspect of the femur. The surface that was closest to the spray apparatus was positioned against the bone. The muscle and skin were closed routinely over the implant in 2 layers. The animals were treated perioperatively with meloxicam (2mg/kg) and buprenorphine (0.06mg/kg) subcutaneously. Seven days after inoculation the rats (n=6, 2 at each 10^2 10^3, and 10^4 CFU/mL) were euthanized and the soft tissue envelope and implants were collected aseptically for microbial quantification. The soft tissue was vortexed for 5 minutes in 5mL of sterile PBS. The implants were washed three times with PBS to remove planktonic bacteria and then sonicated for 10 minutes at 37°C in 0.3% Tween20. The resultant fluids from both implants and soft tissue were then serially diluted, plated on Petrifilms and incubated at 37°C for 24 hours for colony counts.

RESULTS SECTION: In vitro: the highest amount of bacterial attachment to implants was observed at the highest dilution, the 10^4 CFU/mL, which also most closely models operating room contamination. A concentration of 10^2 CFU/mL was found to be too dilute to provide effective bacterial attachment to implants, indicating that further experiments should use a higher strength dilution series. CFU recovery from implants was found to be significantly lower than the input CFU, which is attributable to the reduced surface area of implants compared to spray dispersion. **In vivo:** None of the rats appeared grossly infected, with no overt dehiscence or pockets of purulent material. There was no recoverable bacterial burden on the soft tissue from any of the animals. The recovered CFU/mL from the femoral plates were not statistically different between inoculum concentrations; however, this is likely due to the low cohort number (n=2/concentration). The trend suggests that there is increased variability in the response to the lower inoculum concentrations with 10^4 CFU/mL, resulting in a more reliable and expected recovery. An additional factor is the time between plate contamination and surgical implantation. For the lower two inocula, the plates were sprayed concurrently, but the implants were done consecutively, resulting in a 30-minute time discrepancy between the two samples. The plates that were implanted second both resulted in higher CFU counts, suggesting that when the bacteria are given more time to acclimate prior to insertion, they are more effective at colonizing the implants. The plates inoculated with 10^4 CFU/mL were surgically implanted concurrently, which would explain the similar recovered CFU counts.

DISCUSSION: The model described produces microbial contamination equivalent to that in an operating room, while also allowing for adjustment to produce specific amounts of contamination required to cause infection in 75% of test subjects. This is key to the future study of antimicrobial implant materials in a large animal model, as the goal is to create a consistent infection model to test the antimicrobial properties of the implants without leading to sepsis, a condition that these materials are not designed to combat. In vitro results show that the apparatus reproducibly creates a variety of specified contamination levels, thereby providing a reliable model of operating room contamination. We conclude from this study that the spray apparatus can be used to result in contaminated implants. Further investigation is warranted to determine the optimal time interval between contamination and surgical implantation. Increasing the sample size is necessary to fully understand these trends and is an ongoing process.

SIGNIFICANCE/CLINICAL RELEVANCE: The development of a consistent and adjustable model for operating room contamination enables surgical innovation that supports antimicrobial implant materials. Reproducible contamination of implants leading to reliable infection in rats supports further study in large animal models using the spray apparatus described here.

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