Dual Antibiotic Delivery from Chitosan Sponges Prevents In Vivo Polymicrobial Biofilm Infections

James K. Smith MS[a], Ashley C. Parker MS[a], Jessica A. Jennings PhD[a], Karen E. Beenken PhD[b], Mark S. Smeltzer PhD[b], Warren O. Haggard PhD[a].

[a]The University of Memphis Department of Biomedical Engineering

[b]The University of Arkansas for Medical Sciences Department of Microbiology

Statement of Purpose: The involvement of bone and other compromised tissues, along with the frequent necessity for the use of fracture fixation hardware in battlefield trauma, create conditions where biofilm infections can progress to osteomyelitis. Two of the most problematic bacterial strains in osteomyelitis are *Staphylococcus aureus* and *Pseudomonas aeruginosa*; osteomyelitis treatment failure rates are higher when either *S. aureus* or *P. aeruginosa* are the infecting organisms.[1-3] Another typical complication of a clinical biofilm-based infection is the presence of multiple bacterial and/or fungal strains; therefore, the traditional view of individual pathogen infection treatment may not be appropriate. A local drug delivery system may overcome some of the challenges associated with biofilms, such as limited antibiotic effectiveness, antibiotic delivery to avascular wound areas. The chitosan sponge has previously been established as an effective local antibiotic delivery vehicle and has shown to prevent *S. aureus* biofilm infection *in vivo*.[4-5] Our objective in this study is to assess the capability of a buffered chitosan sponge to locally delivery both vancomycin (V) and amikacin (A) for the prevention of *S. aureus* (UAMS-1) and *P. aeruginosa* (ATCC 27317) biofilm infections in an established *in vivo* infected catheter murine model.[6]

Methods: Chitosan sponges were manufactured by dissolving 1% (w/v) in a 1% (v/v) acid solvent and casting the solutions into containers. Sponges were frozen at -20°C, lyophilized, neutralized in 0.6 M sodium hydroxide, and washed with copious amounts of water. The neutralized and hydrated sponge was soaked in 0.25 M acetate buffer at a pH of 5.6 for 30 minutes. Excess buffer was removed and the sponge was frozen again at -20°C and lyophilized. *In vivo* analysis of the sponges for the prevention of biofilms was conducted using an established biofilm murine model.[6] In this model, two 1 cm pieces of 14 gauge Teflon catheters were implanted under the skin of the mice. An 8 mm diameter piece of the sponge, loaded either with either 1 x PBS, 4 mg/ml V, 4 mg/ml A, or both A and V at 4 mg/ml each, was placed adjacent to each catheter segment (n = 12 per group). The mice were inoculated with a monospecies of 10^5 colony forming units (CFUs) of S. *aureus*, 10^4 CFUs of *P. aeruginosa*, or a mixed infection of both bacterial strains by injection into the lumen of the catheter. The incisions were then covered with intact skin and closed with surgical glue. After 48 hours, the implanted catheters were removed and sonicated to remove adherent bacteria. Serial dilutions of each sample were plated on the appropriate medium to obtain quantitative colony counts. Bacterial quantities (CFUs) were analyzed using ANOVA with Tukey's *post hoc* analysis.

Results: After 48 hours of prophylactic treatment, nearly 100% of *P.aeruginosa* was cleared when exposed to A loaded sponges and A and V loaded sponges in mono and mixed infections, respectively (Fig. 1). All antibiotic treatment groups exhibited less *S. aureus* growth, as compared to the PBS control groups.

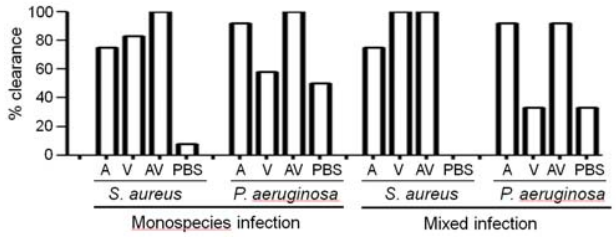

Figure 1. % of catheters with cleared infection from mice with implanted chitosan sponges (n = 12). *S. aureus* and *P . aeruginosa*, as a monospecies infection and in mixed infection, were treated using sponges loaded with vancomycin (V), amikacin (A), both vancomycin and amikacin (AV), or phosphate buffered saline (PBS).

Conclusions: Results indicate that a combination therapy of amikacin and vancomycin released from chitosan sponges does prove effective at preventing *S. aureus* and *P. aeruginosa* biofilm growth, but has a slightly stronger effect on *P. aeruginosa* than *S. aureus*. As expected, single loaded amikacin sponge groups exhibited some activity against *S. aureus* whereas single loaded vancomycin sponges did not have a discernible effect on *P. aeruginosa* growth. A major limitation of this study is the distance of antibiotic diffusion required for the treatment to reach the lumen of the catheter from the sponges; lower antibiotic concentrations might be sufficient in a wound where the sponge is closer to the infection site. Additionally, this *in vivo* study assesses biofilm growth prevention, not treatment of established biofilms. Future *in vivo* murine models will determine if dual antibiotic delivery from the chitosan sponges can reduce or eliminate an established bacterial biofilm on catheters. The results of this study helped to establish a polymicrobial biofilm infected murine model, useful for future infection prevent studies. Additionally, the study indicated that the dual antibiotic loaded chitosan sponges are capable of preventing co-culture biofilm infections in Swiss mice, proving its potential for clinical applications.

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