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

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Introduction: 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 more problematic bacterial strains in osteomyelitis are Methicillin resistant Staphylococcus aureus (MRSA) and Pseudomonas aeruginosa; osteomyelitis treatment failure rates are higher when either MRSA 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, and systemic drug delivery, such as 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 deliver both vancomycin and amikacin 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: Large chitosan sponges were manufactured by dissolving 1% (w/v) in a 1% (v/v) blended acid solvent (3:1 lactic: acetic acid) and casting 250 ml of the solutions into 11 x 20 cm containers. Sponges were frozen at -20°C, lyophilized, and neutralized in 0.6 M sodium hydroxide. The neutralized sponges were 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, 1 mg/ml vancomycin, 4 mg/ml amikacin, or both 1 mg/ml vancomycin and 4 mg/ml amikacin solutions, was placed adjacent to each catheter segment (n = 12 per group). The mice were inoculated with either 10^5 colony forming units (CFUs) of S. aureus, 10^4 CFUs of P. aeruginosa, or a co-culture 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 of either S. aureus or P. aeruginosa. Bacterial quantities (CFUs) were analyzed using ANOVA with Tukey’s post hoc analysis.

Results: After 48 hours of prophylactic treatment, 100% of P. aeruginosa was cleared when exposed to amikacin loaded sponges and vancomycin and amikacin loaded sponges in mono and co-cultures, respectively (Fig. 1a). Amikacin treatment groups, both single and dual loaded, showed significantly decreased P. aeruginosa CFUs in comparison to single loaded vancomycin groups and the control PBS groups (Fig. 1b). All antibiotic treatment groups exhibited significantly less S. aureus growth, as compared to the PBS control groups. More breakthroughs could be seen in S. aureus growth than in P. aeruginosa growth, as illustrated by a 50-58% clearance of S. aureus in the single loaded vancomycin group and reduced clearance percentages of S. aureus compared to P. aeruginosa in amikacin and dual loaded treatment groups.

Discussion: 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. Interestingly, when vancomycin and amikacin were combined, the effect on the S. aureus mono-cultures was weakened, as compared to amikacin alone. Based on the low infection clearance of S. aureus with vancomycin alone, it is clear that the vancomycin concentration is not high enough to clear all of the bacteria. Another co-culture/dual treatment prophylactic study will be conducted in the future where the vancomycin concentration will be raised to 4 mg/ml. 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 are planned to determine if dual antibiotic delivery from the chitosan sponges can reduce or eliminate an established bacterial biofilm on catheters.

**Significance:** The results of this study helped to establish a polymicrobial biofilm infected murine model, useful for future infection prevention studies. Additionally, the study indicated that the dual antibiotic loaded chitosan sponges are capable of preventing co-culture biofilm infections in Swiss mice, showing its potential for clinical applications.

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**References:**
(A) Clearance of Infection from Catheters by Antibiotic-Loaded Sponge after 48 Hour Prophylactic Treatment (n = 12)

(B) Colony Forming Units Grown after 48 Hour Prophylactic Treatment with Antibiotic-Loaded Chitosan Sponge (n = 12)