Antibiotic-loaded chitosan sponges prevent biofilm formation by MRSA in an established mouse catheter model

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Statement of Purpose: Staphylococci are the leading cause of infection in the hospital and are of increasing concern in the healthcare community. An opportunistic pathogen, *Staphylococcus aureus* causes infection after gaining access to the host through breaches in the skin. The increase of resistant strains of *S. aureus* has made treatment of these infections difficult. Also, *S. aureus* infections associated with indwelling devices can be very difficult to treat due to the recalcitrant nature of bacterial biofilms to conventional antibiotics. Prophylactic treatment of indwelling devices with a degradable, local drug delivery system may help prevent biofilm formation and subsequent infection. Chitosan is a well-known, well-researched biocompatible polymer. Chitosan has been shown to be effective at providing a resorbable matrix to deliver therapeutic agents1,2. We investigated the ability of a chitosan sponge loaded with vancomycin, daptomycin, or linezolid to prevent biofilm formation from a USA300 strain of MRSA on an indwelling catheter in an established mouse model. We hypothesized that prophylactic treatment with loaded chitosan sponges would prevent biofilm formation on an indwelling catheter.

Methods: Sponge preparation: Chitosan solution was prepared by dissolving 5.0 grams (g) of chitosan into 500 milliters (ml) of 1% (v/v) acidic solvent. The chitosan used was 71% deacetylated (DDA) from Primex (Iceland). 25 ml of aqueous chitosan was cast into aluminum dishes and frozen for one hour at -80^{o}C. The samples were then lyophilized for 48 hours and neutralized in sodium hydroxide, followed by washing in distilled water. Samples were re-frozen and lyophilized, cut into 6 mm diameter disks and were sterilized via low-dose gamma irradiation (25-32 kGy).

Catheter model: Biofilm formation was assessed *in vivo* using an established murine model3. Chitosan sponges were submerged in 125 microliters of antibiotic solution (vancomycin, linezolid, or daptomycin) or 1x PBS and placed into a subcutaneous pocket created in each hind flank of NIH Swiss mice (n=12). The concentration of each drug was 10x the Clinical and Laboratory Standards Institute (CLSI)-defined breakpoint MIC. A 1- cm fluorinated ethylene propylene (FEP) catheter segment was placed the created pocket. After implantation, the lumen of the catheter was inoculated with 10^5 CFU of a USA300 MRSA strain contained in 100 µl of PBS. After 48 h, the catheters were removed aseptically and rinsed in sterile PBS to remove non-adherent bacteria. Quantitative bacterial cultures were done to determine total number of colonizing bacteria.

Results:

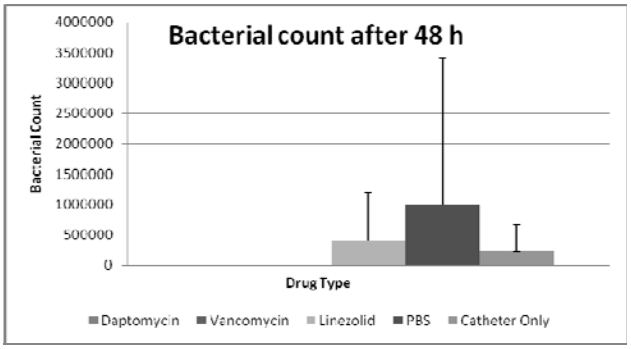

Fig. 1: Graph detailing the bacterial counts of USA300 MRSA after 48 h post-implantation. There were no bacteria present in the groups treated with daptomycin and vancomycin. The groups with linezolid, PBS only ,and positive controls of catheter only were found to have statistically indifferent data. (n=12)

Bacterial counts were determined to be zero for both the daptomycin and vancomycin groups. Groups treated with linezolid and PBS were not found to be statistically different than positive controls of catheter only. The groups treated with vancomycin and daptomycin were statistically different from the other three groups ($p<0.0001$).

Discussion: Chitosan is a well-studied biocompatible polymer that has been used in localized drug delivery applications. Chitosan is currently being used by the United States military as a haemostatic wound dressing material[3]. This study tested the ability of lyophilized chitosan sponges to be used as prophylactic treatment method in an established mouse model using a relevant strain of MRSA. Chitosan sponges allow for customizable treatment regimens in a degradable matrix. Dosing and drug choice are clinician determined and these sponges are loaded at the point of care. The sponges are also customizable in terms of degradation as manufacturing alterations can change degradation rates. Current studies involve evaluation of this technology in animal models assessing degradation, local tissue response, and bacterial eradication from the wound site in addition to characterization as a local drug delivery system. The results presented in this study offer evidence that incorporation of antibiotics into chitosan can potentially provide a local drug delivery system that can be used in conjunction with other prophylactic modalities.

References:

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2.Noel S, et al. Clin Orthop Relat Res. 466(6):1377-82
3. Smeltzer M, et al. Antimicrob Agents Chem Oct 2009, p4096-4102.

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