Evaluation of blended chitosan/polyol injectable paste in an *in vitro* model of osteomyelitis

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Statement of Purpose: Musculoskeletal infections are a serious concern in orthopedics, increasing morbidity, mortality, and treatment difficulty. Osteomyelitis can occur after a traumatic injury, periprosthetic joint replacement, or bone reconstruction. Orthopaedic defects requiring metal screws and fixators are at particular risk of infection due to biofilm formation on implanted biomaterials. Biofilm is intrinsically less susceptible to antibiotic therapy due to decreased metabolic rates and the dormant persister cell phenotype. Mannitol, a sugar alcohol, has been shown to activate bacterial metabolism, reversing the persister cell phenotype within biofilm and increasing susceptibility of biofilm to aminoglycosides [1]. Blends of mannitol and chitosan combine the anti-biofilm properties of mannitol with chitosan drug-delivery systems, which have previously shown to be effective in the treatment of musculoskeletal injuries in the form of an injectable paste [2]. This injectable glycobiomaterial system may prevent biofilm formation and treat existing biofilms by delivering antibiotics while simultaneously increasing bacterial susceptibility to antibiotics. The goals of this project were to evaluate chitosan/mannitol blends for efficacy in preventing *Staphylococcus aureus* biofilm formation in an in vitro tissue model using cadaveric rabbit bone.

Methods: Mannitol was added in 2 or 0% (control) (weight/vol) to 1% (weight/vol) chitosan, 1% (weight/vol) polyethylene glycol solutions in 0.85% (vol/vol) acetic acid. Solutions were frozen, lyophilized, and ground before hydration with a 10 mg/mL amikacin and vancomycin in phosphate buffered saline (PBS) solution at a 2.5 mL/g ratio or PBS alone. Bone was harvested from the femur, tibia, and humerus of New Zealand white female rabbits under aseptic conditions and cut into small 0.5 cm long cylinders. Bone samples were placed into 12 well plates with 500 µL of PBS and inoculated with 100 µL of a 1:10 dilution of *S. aureus* at 10^5 CFU. Pastes (0.3 mL, n = 3) were injected into the medullary canal and on top of the bone sample and incubated overnight at 37°C. A cross-sectional schematic diagram of this set up can be seen in Figure 1 below.

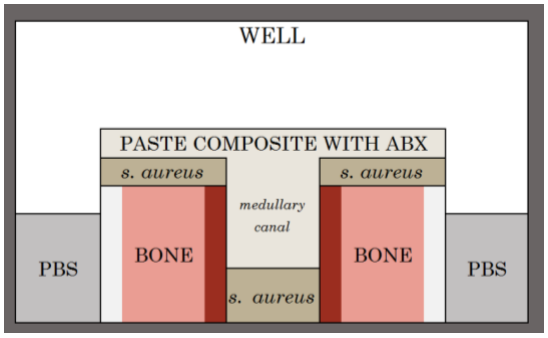

Figure 1. Schematic of Rabbit Bone/Biofilm Model

250 µL was collected from the bottom of the well and used to inoculate a tryptic soy agar (TSA) plate and incubated overnight. After the paste was removed, the bone was placed in 2.50 mL of PBS and sonicated for 5 minutes for biofilm CFU determination.

Results: Paste loaded with the antibiotic solution showed efficacy at preventing biofilm formation (Table 1). The 2% mannitol paste without antibiotics indicated a decrease CFU counts compared to the 0% control with no antibiotics, but the groups were not statistically different.

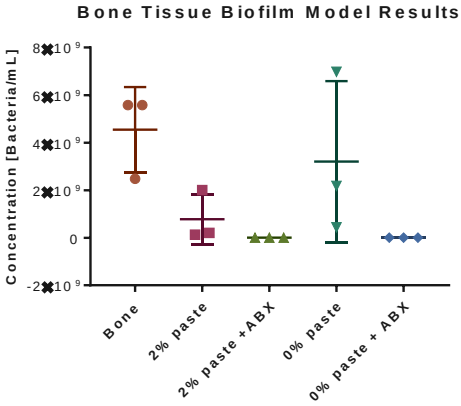

Figure 2. Average concentration of S. aureus for each group based on CFU counts. Paste groups with antibiotics showed complete clearance, while 2% paste without antibiotics showed a reduction in bacteria.

Conclusions: Results indicate that mannitol/chitosan blends were effective at inhibiting biofilm, both with and without antibiotics. This could be attributed to the acidic nature of the paste, or to the inherent antimicrobial activity of chitosan due to its cationic nature. Simple *in vitro* assays of biofilm attached to microtiter plates often lack the complexity of *in vivo* studies to accurately predict the performance of biomaterials in infected models. Future studies will expand evaluations to different bacteria, such as *Pseudomonas aeruginosa*, and to other harvested tissue, such as muscle. Treatment models will also be developed to test the efficacy of the pastes against established biofilm on both bone and muscle prior to *in vivo* infection models.

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