# Prophylactic treatment of *S. aureus* infection using electrospun chitosan membrane in acute osteomyelitis models

Luke J. Tucker[1]; Emily G. Moles[1]; Alyssia J. Little[1]; Ezzuddin E. M. Abuhussein[2]; Joshua R. Bush[2]; J. Amber Jennings[2]; Lauren B. Priddy[1]
[1]Mississippi State University, Mississippi State, MS, USA
[2]University of Memphis, Memphis, TN, USA
Ljt117@msstate.edu

**INTRODUCTION:** Trauma caused by deep penetrating wounds is often complicated by bacterial infection. The standard of care for a puncture wound is to apply a hemostatic technique or material to lessen hemorrhage. The current standard hemostatic material for field medics is Celox™ gauze packed into the wound. While Celox™ can improve survival rates, it lacks an antimicrobial adjuvant which could reduce the risk of infection.[1] Alternatively, use of antimicrobial electrospun chitosan membrane (ESCM) containing cis-2-decenoic acid (C2DA), an antimicrobial fatty acid, and bupivacaine (Bup), a common analgesic with antimicrobial properties may be more effective in addressing this issue. Using an acute model of osteomyelitis, we hypothesized this chitosan-based material packed around a bi-cortical femoral defect infected with *Staphylococcus aureus* (*S. aureus*) would have lower bacterial load compared to the Celox™ standard.

**METHODS:** To develop a prophylactic femoral infection model in the rat for the study of biomaterials-based approaches to mitigate *S. aureus* osteomyelitis, we modified our chronic infection model into a single surgery including infection and treatment. First, we determined the inoculation time necessary to establish repeatable infection via an infected screw in an *ex vivo* femoral rat model. Rat femora were cut transversely in half and a 0.889 mm diameter bi-cortical defect drilled into the diaphysis of each half. Using prior data from our implant-based, chronic osteomyelitis model, orthopedic screws were loaded with ~$1x10^8$ CFU GFP *S. aureus*[2] and placed into the defect for 5 or 15 minutes (n=6) and then removed. Bacterial counts were performed on femora and screws. All *in vivo* procedures were approved by the IACUC. Based on the *ex vivo* data and our chronic infection model,[2] an infected screw was placed into a bi-cortical defect in the left femur of female CD rats[2] for 5 minutes before being removed. Harvested screws were also homogenized and enumerated for colony quantification. 150 mg of either Celox™, ESCM, ESCM soaked in C2DA (35 mg/mL), or ESCM soaked in both C2DA (35 mg/mL) and Bup (40 mg/mL) (n=3) was placed into the soft tissue on the cranial and caudal sides of the femur. Animals were sacrificed at 3 days, and the femur, surrounding soft tissue, and remaining treatment material were collected and homogenized, and colonies were enumerated for quantification. The data was statistically analyzed using either a paired t-test or mixed model in SAS 9.4 (English).

**RESULTS:** No difference in femoral bacterial load between the 5- and 15-minute inoculation groups were observed (**Fig. 1**). Notably, the 5-minute inoculation time had a smaller variance and would minimize time under anesthesia, so it was used for the *in vivo* study. The screws after implantation had similar bacterial levels as those from previous studies. However, the screws from the *in vivo* experiment had bacterial loads at least two orders of magnitude lower than the *ex vivo* and previous *in vivo* experiments. All animals developed acute osteomyelitis. No differences between groups were found in either the bone or remaining treatment material (**Fig. 2**). In contrast, the surrounding soft tissue for both ESCM and ESCM+C2DA had lower bacterial loads than that for the ESCM+C2DA+Bup group, while the bacterial load for ESCM was also lower than the Celox™ control (**Fig. 2**).

**DISCUSSION:** The lower bacterial counts from the recovered screws *in vivo* compared to those from *ex vivo* could be due to the physiological tissue environment promoting more efficient transfer of bacteria from the implant to the tissue. While this model is novel and would benefit from additional characterization, it consistently generated acute osteomyelitis with quantifiable differences in the treatments. The animals recovered quickly from the surgery, and the infection upon gross inspection remained contained to the bone and surrounding soft tissue. The materials were easily recoverable, so as not to affect colony enumeration in bone or soft tissue. Minimal effects between treatments were observed in the bone, but significant effects were measured in the soft tissue. This is similar to prior findings with other treatments in our chronic infection model, as the soft tissue is more accessible to the circulating immune system and is where most of the treatment volume resides.[2] While ESCM +/- C2DA were successful in reducing bacterial burden in soft tissue, the addition of Bup abrogated this effect. This could be due to Bup being a sodium channel blocker which may suppress antibacterial Th1-mediated immune response to a Th2-mediated response.[3] Ongoing work includes the addition of an untreated control and ESCM+Bup groups and increasing the sample size.

**SIGNIFICANCE/CLINICAL RELEVANCE:** There is a critical need for the development of an antimicrobial gauze that can be used on acute wounds to mitigate the risk of infection until the patient can reach a trauma center. Our pilot data shows that chitosan-based materials packed into the soft tissue in a rat model of osteomyelitis significantly decreased bacterial burden when compared to the standard of care Celox™ gauze.

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**REFERENCES:**

**1**. Winstanley, M., et al. *J R Army Med Corps* 2019. **2**. Cobb, L. H. *et al. PLoS One* 2019. **3**. Roselli, F., et al. *Recent Pat CNS Drug Discov* 2006.

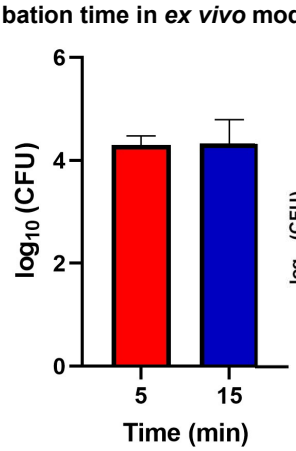


**Figure 1.** No significant difference in CFU in the bone due to incubation time (n=6, p=0.8376)

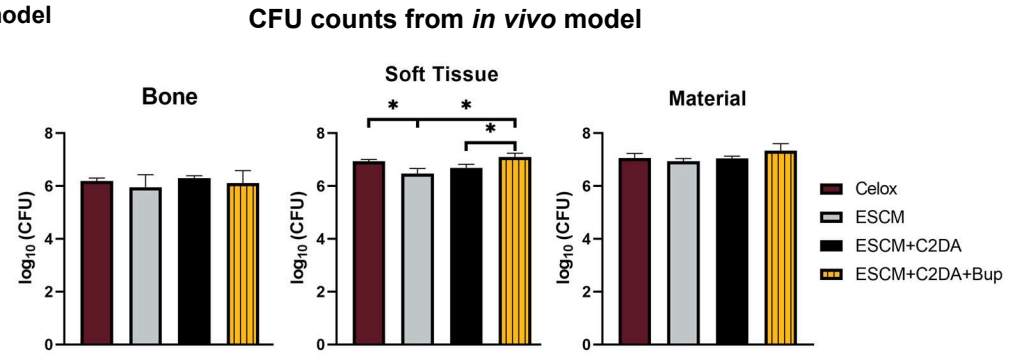


**Figure 2.** After three days, tissues were collected from the animals and the resulting colonies were enumerated. No differences were observed in either the bone or recovered treatment materials. The surrounding soft tissue had a significant difference in treatments, with pairwise differences between Celox™ and ESCM, ESCM and ESCM+C2DA+Bup, and ESCM+C2DA and ESCM+C2DA+Bup (*p<0.05, n=3).