Animal Model of Open Femur-Fracture Infection Using Noninvasive *In Vivo* Bioluminescence Imaging

+Li, B; Farjo, P; Hamza, T
Department of Orthopaedics, School of Medicine, West Virginia University, Morgantown, WV, USA
bili@hsc.wvu.edu

INTRODUCTION: Open fracture-associated infection is a devastating problem faced by millions of patients worldwide. *Staphylococcus aureus* (*S. aureus*) is a leading cause of such infections. **The aim of this study** was to establish an open fracture infection animal model that allows noninvasive monitoring of bacterial burden using bioluminescent *S. aureus*. To establish such a model, we investigated different bacterial concentrations and the bacterial burdens were monitored till euthanization of the animals at post-operative day 21.

HYPOTHESIS: We hypothesized that the use of bioluminescent *S. aureus* would allow monitor infection temporally and spatially for a long time period using a noninvasive *in vivo* bioluminescence imaging technology.

METHODS: An open femur fracture was created using Sprague-Dawley rats. The rats' femurs were fractured, inoculated with 100 µL bioluminescent *S. aurues* (Xen36, Caliper Life Sciences, Alameda, CA), left open for one hour, and then fixed with a stainless steel Kirschner wire (K-wire). Xen36 was chosen because it had shown stable bioluminescent constructs in other animal models. A total of three groups were studied (six rats per group): Group A - 10^2 colony forming units/mL or CFU/mL, Group B - 10^4 CFU/mL, and Group C - 10^6 CFU/mL. Animals were anesthetized at post-operative days 3, 7, 10, 14, and 21 and quantified for *in vivo S. aureus* burden using the Xenogen IVIS Lumina Imaging System. Meanwhile, radiographs for each sample were taken at post-operative days 0, 7, and 21, and body weight assessments were taken for each sample at post-operative days 0, 7, 10, 14, and 21. Animals were euthanized on post-operative day 21, and samples of blood, muscle, bone, and K-wire were collected to determine infection and systemic responses. The K-wire was collected, and a 2-cm piece was cut sterilely and rolled on a blood agar plate followed by incubation at 37°C for 24 h; the rest of the K-wire was processed and examined under scanning electron microscopy (SEM). Histology samples were also placed in 10% formalin and processed.

RESULTS AND DISCUSSION: We developed an open femur fracture rat infection model using bioluminescent *S. aureus*. Quantitative culturing of bone and muscle tissue homogenates at post-operative day 21 found that the three animal groups all had approximately 10^7 CFU/gram of bone tissue and 10^6 CFU/gram of muscle tissue, respectively. K-wire rolling experiments also showed numerous colonies of *S. aureus*, and SEM examination presented biofilms on K-wires with numerous colonies embedded within polymer matrixes (**Fig. 1**). However, IVIS imaging showed no bioluminescent signal in Group A, and the bioluminescent signals in Group B peaked on post-operative day 7 followed by a decrease on day 10 and disappearance by days 14 and 21 (**Fig. 2**). Since biofilms were observed on all the K-wires and high CFUs were cultured from bone and muscle samples at post-operative day 21, these data suggest that the bioluminescent construct was lost during *in vivo* replication in the absence of antibiotic selection. Meanwhile, the bioluminescent signals were detectable at and before post-operative day 10 in Groups B and C therefore may be useful for early stage infection detection. Interestingly, by superimposing IVIS and radiographical images of the same animal, we could estimate the distribution of *S. aureus* in the fracture model, and we found that more bacteria were located at the distal femur (**Fig. 3**).

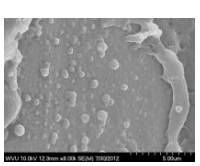

Fig. 1. Biofilm observation.

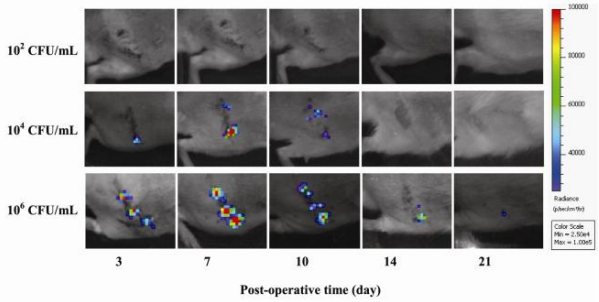

Fig. 2. Representative *in vivo* bioluminescence signals of Xen36 in an open femur rat fracture model.

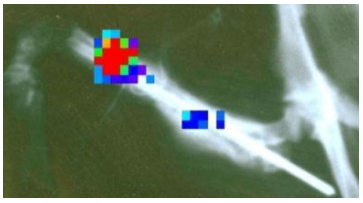

Fig. 3. Bacterial distribution profile in Group B at post-operative day 7 obtained by superimposing IVIS and radiographical images.

CONCLUSIONS: (1) We established an open femur fracture infection model using bioluminescent *S. aureus*, which allowed the determination of *S. aureus* distribution in the fracture model. (2) Bioluminescence was used to detect active bacterial colonies and the *in vivo* signals in Group B peaked at post-operative days 7 to 10. (3) Noninvasive bioluminescence imaging may have the potential to monitor infection in animal models for long-term study. However, challenges exist.

SIGNIFICANCE: The establishment of a noninvasive *in vivo* imaging technology may be very valuable for future studies of the pathogenesis of open fracture-associated infections, because it may allow for measurement of bacterial burden without euthanizing animals to collect tissue and implant samples and may offer visualization of temporal and spatial progression of infection in real time.