Mouse Model of Chronic Post-Arthroplasty *Staphylococcus aureus* Infection: Determination of the Optimal Bioluminescent Bacterial Strain for Long-Term Study

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INTRODUCTION:

Over the next 20 years, the number of joint replacements performed annually in the U.S. is projected to increase from 600,000 to more than 4 million. Postarthroplasty infections occur in ~0.5-1% of primary joint replacements and 3-5% of revisions. These infections are clinically devastating, leading to extended rehabilitation, increased health care costs, and significantly worse clinical outcomes. In our previous work, we developed a mouse model of postarthroplasty *Staphylococcus aureus* infection using in vivo bioluminescence imaging on monitor bacterial burden in real-time over the course of 10 days and assess the efficacy of an antimicrobial polymer coating in the prevention of the infection. In the present study, we aimed to develop a mouse model of postarthroplasty *S. aureus* infection that would persist 6 weeks after surgery to more closely model a chronic postarthroplasty infection. To accomplish this, we compared the previously utilized SH1000 bioluminescent strain (that contains the bioluminescent lux operon on a chloramphenicol selection plasmid) with Xen29 and Xen36 bioluminescent strains (bioluminescent lux operon embedded in the bacterial chromosome or within a stable plasmid, respectively) to determine which *S. aureus* strain would provide the most consistent and accurate bioluminescent signals up to 6 weeks post-operatively. With an appropriate *S. aureus* strain, this model of chronic, long-term bacterial postarthroplasty infection would provide researchers a tool with which to examine the long-term efficacy of antimicrobial and antibacterial interventions, as well as the immunologic responses to infection and biofilm formation.

![Figure 1. Radiograph demonstrating retrograde placement of the implant into the femur with intrarticular extension.](image)

METHODS:

All procedures were approved by the UCLA Animal Research Committee (ARC#: 2008-112). 12-week old male mice on a C57BL/6 background were used in all experiments. To simulate a postarthroplasty infection, the distal right femur was accessed through a medial parapatellar arthrotomy with lateral displacement of the quadriceps-patellar complex. An orthopaedic-grade stainless steel K-wire (diameter 0.6 mm) was placed in a retrograde fashion into the femur and cut with 1 mm protruding into the joint space (Figure 1). Three strains of *S. aureus* (bioluminescent SH1000 strain [lux operon on a chloramphenicol selection plasmid], Xen29 strain [lux operon integrated into the chromosome], and Xen36 strain [lux operon in a stable plasmid] [Xenogen, Caliper Life Sciences]) (1e3 CFUs) were diluted in a 2 µl volume and inoculated into the joint space. Bacterial burden within the infected postoperative knee joints was measured with in vivo bioluminescence imaging on postoperative days 0, 1, 3, 5, 7, 10, 14, 21, 28, 35, and 42 (Fig 2). Bioluminescence was correlated to bacterial burden with bacterial colony counts at serial time points. Biofilm formation was assessed using variable pressure scanning electron microscopy (VP-SEM). Statistical differences between groups were determined by a Student’s t test (one-tailed).

![Figure 2. In vivo bioluminescent imaging of *S. aureus* within the infected post-operative knee joints.](image)

DISCUSSION:

Whole small animal imaging is a new technology that allows an innocuous and real-time measurement of bacterial burden and biofilm formation on implanted materials. In the SH1000 strain, which was utilized in our previous work, the bioluminescent lux operon is incorporated into a plasmid that has chloramphenicol resistance and, in vivo, it is likely that this plasmid will be lost over time. In contrast, the Xen29 strain has the bioluminescent lux operon in the chromosome and Xen36 has the bioluminescent lux operon in a stable plasmid that does not require antibiotic selection to maintain the operon. We found that the Xen36 strain had the highest bacterial signals that persisted above the background signals through day 42. The signals with the Xen29 strain with the lux operon in the chromosome were also detectable, but were just above the level of the background signals. In contrast, the SH1000 strain had signals that were not significantly greater than background levels after 14 days. Based on these data, it is clear that the use of a *S. aureus* strain with a more stable lux operon construct, either in a stable plasmid or within the bacterial chromosome, provides a better approximation of bacterial burden beyond 14 days. A bacterial strain that will continue to produce a detectable signal over a 6 week infection course will allow us to establish and monitor a chronic postarthroplasty infection. This model will allow the investigation of immune responses to chronic postarthroplasty infection and the evaluation of antibiotic and antimicrobial coatings that may protect against bacterial infection after total joint replacement.