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Preclinical Models and Methodologies for Monitoring *Staphylococcus aureus* Infections Using Noninvasive Optical Imaging

Nathan K Archer¹, Yu Wang¹, Roger V Ortines¹, Haiyun Liu¹, Sabrina J Nolan¹, Qi Liu¹, Martin P Alphonse¹, Dustin A Dikeman¹, Momina Mazhar¹, Robert J Miller¹, Leif S Anderson², Kevin P Francis³, Scott I Simon², Lloyd S Miller^{4,5,6,7}

¹Department of Dermatology, Johns Hopkins University School of Medicine, Baltimore, MD, USA.

²Department of Biomedical Engineering, University of California, Davis, Davis, CA, USA.

³PerkinElmer, Inc., Hopkinton, MA, USA.

⁴Department of Dermatology, Johns Hopkins University School of Medicine, Baltimore, MD, USA.

⁵Department of Medicine, Division of Infectious Diseases, Johns Hopkins University School of Medicine, Baltimore, MD, USA.

⁶Department of Orthopaedic Surgery, Johns Hopkins University School of Medicine, Baltimore, MD, USA.

⁷Department of Materials Science and Engineering, Johns Hopkins University, Baltimore, MD, USA.

Abstract

In vivo whole-animal optical (bioluminescence and fluorescence) imaging of *Staphylococcus aureus* infections has provided the opportunity to noninvasively and longitudinally monitor the dynamics of the bacterial burden and ensuing host immune responses in live anesthetized animals. Herein, we describe several different mouse models of *S. aureus* skin infection, skin inflammation, incisional/excisional wound infections, as well as mouse and rabbit models of orthopedic implant infection, which utilized this imaging technology. These animal models and imaging methodologies provide insights into the pathogenesis of these infections and innate and adaptive immune responses, as well as the preclinical evaluation of diagnostic and treatment modalities. Noninvasive approaches to investigate host-pathogen interactions are extremely important as virulent community-acquired methicillin-resistant *S. aureus* strains (CA-MRSA) are spreading through the normal human population, becoming more antibiotic resistant and creating a serious threat to public health.

Keywords

Imaging; Optical; Bioluminescence; Fluorescence; *Staphylococcus aureus*; Infection; Inflammation; Immune; Bone; Orthopedic; Implant; Biofilm; Skin; Wound

1 Introduction

In vivo whole-animal bioluminescence imaging (BLI) and fluorescence imaging (FLI) are noninvasive approaches that detect and visualize photons of light. For in vivo BLI, the photons arise from a luciferase-driven, enzymatic reaction. For in vivo FLI, the photons arise after excitation of a fluorescent molecule or protein that results in the emission of a longer wavelength of fluorescent light [1–4]. To facilitate the detection of light, in vivo BLI and FLI typically involve using in vivo imaging systems (IVIS) with dark chambers in which anesthetized animals can be placed and either bioluminescence- or fluorescence-generated photons are detected using highly sensitive photon detectors, such as cooled (and intensified) charge-coupled device (CCD) cameras (Fig. 1) [1–4].

These techniques enable the visualization and quantification of the intensity and the spatial and anatomical localization of the light passing through different tissue types in live animals, including skin, subcutaneous tissues, muscles, bone, and internal organs [1–4]. In addition, the same animals can be visualized at different time points, allowing the dynamics of the light signals to be temporally visualized, which provides noninvasive longitudinal information about the corresponding disease events over time. Therefore, the numbers of animals can be substantially reduced since euthanasia is not required to obtain information about the bacterial burden and/or host immune responses. Moreover, in vivo BLI and FLI techniques promote the refinement of methods to minimize animal pain or distress by providing noninvasive data of various endpoints, such as bacterial burden, localization of the infection, and host immune responses that would otherwise require invasive sampling techniques to obtain blood, tissue, organ, or bodily fluid specimens. Furthermore, the information gained from in vivo BLI and FLI can be used to determine the most relevant time points to perform any additional sampling or experiments on the animals, thereby minimizing the stress or discomfort of performing repeated procedures.

Bioluminescent signals are naturally found in many types of organisms and involve the production of light from luciferase enzymes and a specific luciferin substrate in the presence of energy (e.g., FMNH₂ and ATP) and oxygen [5, 6]. To date, the most applicable bioluminescent system for infectious disease research has been the *lux* operon from the bacterial insect pathogen *Photobacterium luminescens*. This *luxCDABE* operon encodes both the heterodimeric bacterial luciferase enzyme (*luxAB*) and the substrate (*luxCDE*) for light production at a peak wavelength of 490 nm [5, 6]. Modified versions of this *lux* operon have been stably integrated into many different bacterial species, such as *Staphylococcus aureus* for in vivo BLI [7–9] of the preclinical models described in this chapter.

As an alternative approach, microorganisms and eukaryotic cells can be engineered to express luciferase enzymes from other organisms, including those from firefly (e.g., *Photinus pyralis*), click beetle (*Pyrophorus plagiophthalmus*), sea pansy (*Renilla reniformis*), and marine copepod (*Gaussia princeps*) [1, 2, 4–6]. However, the substrates for these enzymes must be exogenously administered for the luciferase enzymes to act as reporters for in vivo BLI. Although exogenously administered luciferin (most commonly via subcutaneous, intraperitoneal, or intravenous routes) is well distributed throughout the

animal and substrate availability is typically not a limiting factor for small animal in vivo BLI, there is a defined time window for image acquisition corresponding to the optimal in vivo substrate-luciferase interaction. This is not an issue with using the *lux* operon because the administration of an exogenous substrate is not required. Eukaryotic luciferases also require higher oxygen tensions than bacterial luciferases to function optimally. However, there are advantages of using luciferase enzymes from firefly or click beetle, even though they require exogenously administered luciferin. For example, it is only necessary to engineer the microorganisms with a single-gene construct, making the transformation process somewhat simpler than engineering microorganisms with the five-gene *lux* operon of *Photobacterium luminescens*. Moreover, the emitted bioluminescent light produced by firefly and click beetle luciferases has a significantly longer wavelength (peak 600 nm), which permits deeper tissue penetration of the signal compared to the shorter wavelength (peak 490 nm) light produced by the *lux* operon.

Fluorescent compounds have an inherent property in which irradiation at a specific “excitation” wavelength of light results in excitation of an electron in the molecule to a higher energy state, followed by the return of the electron to a lower energy state that results in the “emission” of red-shifted fluorescent light [1–4]. Numerous fluorescent compounds, such as green fluorescent protein (GFP) from the *Aequorea victoria* jellyfish [10] and DsRed protein from the coral *Discosoma species* [11], are available for in vivo FLI. This chapter highlights the use of a transgenic mouse that expresses DsRed fluorescence based on IL-1- β -promoter activity (pIL1-DsRed mice) [12, 13] as well as a knock-in mouse strain with green fluorescent neutrophils (LysM-EGFP mice) (Fig. 2) [12, 14–19]. For a fluorescent compound to perform successfully as an in vivo FLI agent, longer wavelengths are generally more efficient due to their enhanced tissue penetration with red-shifted and near-infrared (NIR) fluorescent compounds (>650 nm). However, in vivo FLI often has inherent problems with sensitivity due to background autofluorescence. Moreover, both the excitation light and the emission light have to penetrate through tissue, and this creates problems due to tissue absorption and scattering of the light [3, 4].

Using optically engineered reporter microorganisms and host animals in combination can provide extremely valuable information regarding the pathogen and host responses that occur during the course of an infection in vivo. Described in this chapter are examples of longitudinal in vivo BLI and FLI imaging protocols in the context of *S. aureus* infection in different animal models, including (1) an intradermal skin infection mouse model [8, 12, 20–26], (2) an epicutaneous skin inflammation mouse model [27–29], (3) an incisional wound infection mouse model [30–32], (4) an excisional wound infection mouse model [15, 17–19], (5) an orthopedic implant infection mouse model [14, 33–41], (6) a hematogenous orthopedic implant infection mouse model [42], and finally (7) an orthopedic implant infection rabbit model [43]. As the technology continues to advance for in vivo optical imaging, the specific techniques and models described herein could be adapted for noninvasive in vivo BLI or FLI in many other animal models of infection.

In our initial studies in the intradermal skin infection mouse model, the incisional mouse wound infection model, and the orthopedic implant infection mouse model, we used the bioluminescent *S. aureus* SH1000 strain ALC2906, which possesses the shuttle plasmid

pSK236 with the penicillin-binding protein 2 (pbp2) promoter fused to the modified *lux* operon reporter cassette from *Photorhabdus luminescens* [8, 14, 15, 17, 18, 20, 21]. This strain emits bioluminescence signals from live, actively metabolizing bacteria in all stages of the *S. aureus* life cycle. Since the *lux* operon in ALC2906 is maintained in a plasmid construct, all cultures on plates and in broth should be performed in the presence of chloramphenicol 10 µg/mL to maintain the plasmid. In additional studies using the intradermal skin infection and the incisional wound infection models, we used the CA-MRSA strain USA300 LAC::*lux* or *SAP231*, which have the bioluminescent construct maintained in the bacterial chromosome and do not require any antibiotic selection [26, 33–36]. This strain was derived from the parent USA300 LAC strain from an outbreak in the Los Angeles County Jail [44]. Most recently, we have used strain LAC4303 in the epicutaneous skin inflammation mouse model [27] and SAP231 in the orthopedic implant infection mouse model, the hematogenous orthopedic implant infection mouse model, and the orthopedic implant infection rabbit model [32, 40–43, 45]. LAC4303 was derived from the parent CA-MRSA LAC strain (described above), and SAP231 was derived from the parent CA-MRSA NRS384 strain that was isolated from an outbreak in the Mississippi Prison System [9]. Both LAC4303 and SAP231 possess the same modified *lux* operon inserted into a pseudogene under the control of a strong constitutively active promoter. Importantly, LAC4303 and SAP231 bioluminescent *S. aureus* strains have at least tenfold greater light production than any of the aforementioned bioluminescent *S. aureus* strains and provided the new capability of detecting in vivo BLI signals from internal organs to noninvasively monitor invasive infections and metastatic bacterial dissemination in anesthetized mice [9, 42]. Similar to USA300 LAC::*lux*, both LAC4303 and SAP231 possess the *lux* construct in the bacterial chromosome and therefore do not require any antibiotic selection when growing or working with the bacteria. Growth conditions are strain specific and should be determined for each of the bioluminescent *S. aureus* strains before they are used in experiments. For all models described below, the methods for preparation of mid-logarithmic bacterial cultures are similar.

2 Materials

2.1 Preparing the Inoculum of a Mid-Logarithmic Bioluminescent *S. aureus* Strain

1. Tryptic soy broth (TSB).
2. Tryptic soy agar (TSA) plates.
3. Blood agar (TSA with 5% sheep blood) plates.
4. Sterile inoculating loops.
5. Sterile phosphate-buffered saline (PBS).
6. Sterile water.
7. Petri dishes.
8. 15 mL Cell culture conical round-bottom tubes.
9. 50 mL Conical centrifugation tubes.

10. 1.5 mL Microcentrifuge tubes.
11. Aerosol-resistant tips for micropipettes (20, 200, and 1000 μ L tips).
12. 5, 10, and 25 mL pipettes.
13. Spectrophotometer.
14. Luminometer.
15. A bioluminescent *S. aureus* strain.

2.2 Materials for Intradermal Skin Infection Mouse Model

The rise of CA-MRSA infections, in particular skin abscesses in the healthy human population, has increased the urgency to develop novel host-directed therapeutic interventions as an alternative to antibiotic therapy [46, 47]. The intradermal skin infection model induces the development of a skin ulcer and robust neutrophil abscess formation in mice (Fig. 2a). This model, in conjunction with in vivo BLI, is widely used to evaluate immune responses, host-pathogen interactions, and therapeutics, which have all been the focus of our efforts (Fig. 2b) [8, 12, 20–26]. The protocol described in Subheading 3.2 is an example of combining in vivo BLI and FLI to monitor bacterial burden and host immune responses during the intradermal skin infection. Specifically, transgenic mice with DsRed expression under the IL-1 β promoter (pIL1-DsRed reporter mice) and EGFP expression in primarily myeloid cells (especially neutrophils) under the control of the lysozyme promoter (LysM-EGFP mice) were used to track IL-1 β expression and neutrophil accumulation at the site of the *S. aureus* skin infection, noninvasively and longitudinally over time (Fig. 2c, d, respectively) [12].

1. 6- to 8-week-old C57BL/6 mice.
2. 6- to 8-week-old pIL1-DsRed reporter mice [13].
3. 6- to 8-week-old LysM-EGFP mice [16].
4. Isoflurane.
5. 29-gauge insulin syringe.
6. Animal clippers (ProClip clippers equipped with size 40 carbon-infused steel blade).
7. Digital camera mounted on a copy stand.
8. Whole-animal in vivo imaging system (Fig. 1).

2.3 Materials for Epicutaneous Skin Inflammation Mouse Model

S. aureus skin colonization is associated with atopic dermatitis flares and disease severity in patients [48, 49]. The epicutaneous infection model mimics the inflamed surface of atopic dermatitis flares by topical exposure of *S. aureus* by applying a gauze pad soaked with an inoculum of *S. aureus* for 7 days onto the shaved and depilated skin of mice (Fig. 3a). The in vivo BLI signals can be measured on the inflamed skin (Fig. 3b), and the affected skin can be homogenized so that the ex vivo CFU in the inflamed skin can be determined (Fig. 3c),

which can be correlated to the in vivo BLI signals. The *S. aureus* epicutaneous infection model allows for investigating the host-pathogen factors that promote *S. aureus*-induced atopic dermatitis-like skin inflammation in mice [27–29].

1. 6- to 8-week-old C57BL/6 or Balb/c mice.
2. Isoflurane.
3. Adhesive bandages.
4. Animal clippers (ProClip clippers equipped with size 40 carbon-infused steel blade).
5. Depilatory cream.
6. Forceps.
7. Sterile cotton gauze pad.
8. Transparent bio-occlusive dressing.
9. Bioluminescent *S. aureus* strain (strain LAC4303, for example [27]).
10. Digital camera mounted on a copy stand.
11. Whole-animal in vivo imaging system (Fig. 1).

2.4 Materials of Incisional Wound Infection Mouse Model

S. aureus is a common pathogen that infects wounds [50], especially diabetic foot ulcers [51, 52]. This *S. aureus* incisional wound infection model is adapted to work on mice with a genetic predisposition to type II diabetes (i.e., TallyHo/JngJ [strain #005314], NONcNZO10/LtJ [strain # 004456], BKS.Cg-Dock7^m +/- Lepr^{db}/J [db/db, strain #000642], etc. [all from Jackson Laboratories, Bar Harbor, ME]) [31, 32]). This protocol provides an example of a *S. aureus* wound infection in TallyHo/JngJ mice (Fig. 4), but can also be used to study *S. aureus* wound infection in other diabetic mouse strains and in nondiabetic mice, such as C57BL/6 mice [30–32]. Using this model, the wound sizes can be measured in *S. aureus*-infected versus -uninfected mice (Fig. 4a, b), and in vivo BLI signals of the bioluminescent *S. aureus* bacteria in the infected wounds can be measured noninvasively and longitudinally over time (Fig. 4c, d).

1. 6- to 8-week-old C57BL/6 mice can be used to study wound infections under normal conditions. Alternatively, this model can be used in 8- to 10-week-old diabetic mice (i.e., TallyHo/JngJ [strain #005314], NONcNZO10/LtJ [strain # 004456], BKS.Cg-Dock7^m +/- Lepr^{db}/J [db/db, strain #000642], etc. [53]).
2. Isoflurane.
3. Glucometer.
4. Animal clippers (ProClip clippers equipped with size 40 carbon-infused steel blade).
5. Bioluminescent *S. aureus* (strain SAP231, for example [9, 32]).
6. Whole-animal in vivo imaging system (Fig. 1).

2.5 Materials for Excisional Wound Infection Mouse Model

S. aureus is one of the most common pathogens that infect human wounds and contribute to delayed wound healing [50, 54]. In our previous work, we demonstrated that using bioluminescent *S. aureus* bacteria in conjunction with LysM-EGFP mice and performing sequential in vivo BLI and in vivo FLI can study the dynamics of neutrophil infiltration to sterile and *S. aureus*-infected excisional wounds noninvasively and longitudinally over time [15, 17–19]. This model provides an example of a *S. aureus* excisional wound infection in LysM-EGFP mice (Fig. 5).

1. 6- to 12-week-old LysM-EGFP mice on a C57BL/6 background can be used to study wound infections under normal conditions. Alternatively, this model can be used in 8- to 10-week-old diabetic mice (i.e., TallyHo/JngJ [strain #005314], NONcNZO10/LtJ [strain # 004456], BKS.Cg-Dock7^m +/+ Lepr^{db}/J [db/db, strain #000642], etc. [53]).
2. Bioluminescent *S. aureus* strain (see Subheading 2.1, above).
3. Isoflurane.
4. 10% w/v Povidone-iodine.
5. 70% Ethanol.
6. Animal clippers (ProClip clippers equipped with size 40 carbon-infused steel blade).
7. 6 mm Punch biopsy tool.
8. 28-gauge insulin syringe (0.5 ml).
9. Whole-animal in vivo imaging system (Fig. 1).

2.6 Materials for Orthopedic Implant Infection Mouse Model

S. aureus-associated orthopedic implant infections, especially periprosthetic joint infections, represent a serious complication in orthopedic surgery [55–57]. This mouse model of a *S. aureus* orthopedic implant infection involves many aspects of human orthopedic implant infections, including the development of septic arthritis, osteomyelitis, and biofilm formation on an orthopedic implant [14, 33–41]. Therefore, this model has been used in conjunction with in vivo BLI (Fig. 6a, b) and in vivo FLI of LysM-EGFP mice (Fig. 6c, d) to monitor the course of infection, evaluate immune responses (such as neutrophil infiltration in the LysM-EGFP mice), and determine the preclinical efficacy of prophylactic and treatment strategies as well as novel bacteria-specific imaging probes [14, 33–41].

1. 10- to 12-week-old male C57BL/6 mice (see Note 1).
2. 10- to 12-week-old male LysM-EGFP mice (see Note 1).
3. Isoflurane.
4. Sustained-release buprenorphine (2.5 mg/kg).
5. Povidone-iodine Prep Pads.

6. Alcohol Prep Pad.
7. 25-gauge needle.
8. Disposable scalpel (#11 blade).
9. Vicryl 5–0 sutures.
10. Orthopedic-grade titanium Kirschner-wire (K-wire, 9 mm length, 0.6 mm diameter).
11. Bioluminescent *S. aureus* strain.
12. Whole-animal in vivo imaging system (Fig. 1).

2.7 Materials for Hematogenous Orthopedic Implant Infection Model

Although orthopedic implant infections most often occur from bacteria invading the surgical site either during surgery or in the immediate postoperative period, bacteria can also seed a previously sterile and well-functioning implant hematogenously following a transient bacteremia [58–60]. Therefore, we developed a hematogenous orthopedic infection model by modifying the orthopedic infection model described above, such that the bacteria are inoculated intravenously 3 weeks after implantation [42] rather than at the time of surgery (Fig. 7a). This model used the brighter *S. aureus* bioluminescent strain SAP231 (see Subheading 2.1, above), in which in vivo BLI signals can be detected from bacteria in internal organs and from bacteria that hematogenously spread to the surgical leg with the implant that was previously placed in the right femurs of the mice (Fig. 7b). This protocol also provides an example of how ex vivo BLI can also be used to determine the bacterial burden and location of infection in specific organs and in the postsurgical legs of the mice (Fig. 7c).

1. 10- to 12-week-old male C57BL/6 mice (see Note 1).
2. Isoflurane.
3. Sustained-release buprenorphine (2.5 mg/kg) (ZooPharm).
4. Povidone-iodine Prep Pads.
5. Alcohol Prep Pad.
6. 25-gauge needle.
7. Disposable scalpel (#11 blade).
8. Vicryl 5–0 sutures.
9. Orthopedic-grade titanium Kirschner-wire (K-wire, 9 mm length, 0.6 mm diameter).
10. Bioluminescent *S. aureus* strain (strain SAP231, for example [42]).
11. Whole-animal in vivo imaging system (Fig. 1).

2.8 Materials for Orthopedic Implant Infection Rabbit Model

To provide a better representation of the surgical procedures and implants used in orthopedic surgery in humans, we recently developed a rabbit model of orthopedic implant *S. aureus* infection [43]. The surgical procedure involves a similar surgical approach and layered closure of a medial parapatellar arthrotomy in humans. Moreover, a threaded implant could be used to stabilize the implant within the intercondylar bone, which represents improvements over mouse and rat models of orthopedic implant infections [61]. In addition, *S. aureus* virulence factors, such as Panton-Valentine leukocidin (PVL), have activity in rabbits but not mice [62]. Therefore, a rabbit model of orthopedic implant infection that employs in vivo BLI could greatly enhance the translation of findings in preclinical animal models to humans [61] and the protocol for our rabbit model is described below. The procedure for the rabbit model of orthopedic implant *S. aureus* infection involves drilling a hole in the distal femur, inoculating the bioluminescent *S. aureus* bacteria and inserting a threaded locking peg with a screwdriver retrograde into the femur (Fig. 8a). To provide the opportunity to detect in vivo BLI signals from the deep tissue of a rabbit leg, a bright *S. aureus* bioluminescent strain (e.g., SAP231) was used (Fig. 8b). In addition, this model was performed in smaller Dutch-Belted rabbits (~2 kg body weight), which allowed them to be placed within the standard chamber of a commercial IVIS (Lumina III, PerkinElmer).

1. 10- to 16-week-old male Dutch-Belted rabbits (~2 kg body weight).
2. Isoflurane.
3. Ketamine (25 mg/kg).
4. Xylazine (1.5 mg/kg).
5. 10% w/v Povidone-iodine.
6. 70% Alcohol pads.
7. 25-gauge needle.
8. Scalpel blade (#11).
9. Vicryl 5–0 sutures.
10. Orthopedic-grade titanium locking pegs (2 mm × 24 mm).
11. Electric drill equipped with 2 and 2.2 mm drill bits.
12. Sustained-release buprenorphine (0.2 mg/kg).
13. Sustained-release meloxicam (0.6 mg/dose).
14. Metoclopramide (0.3 mg/kg).
15. Bioluminescent *S. aureus* strain (see Subheading 2.1, above).
16. Whole-animal in vivo imaging system (Fig. 1).

2.9 Materials for Ex Vivo CFU Enumeration

The in vivo BLI detected from each of the animal models can be correlated with the actual in vivo bacterial burden by enumerating ex vivo CFU from tissue and organ specimens from all

of the models as well as from the implants in both of the orthopedic implant infection mouse models. BLI of the ex vivo internal organs (liver, kidney, heart, spleen, etc.) can also be conducted after euthanasia of the mice by placing the organs in 6- or 12-well plates to evaluate for organ dissemination. These procedures are modified to enumerate ex vivo CFU in the orthopedic implant infection rabbit model because the tissue specimens and implants are larger.

1. 70% Ethanol.
2. Tryptic soy broth (TSB).
3. Tryptic soy agar (TSA) plates.
4. Sterile phosphate-buffered saline (PBS).
5. Tween 20.
6. 2 mL Cryogenic vial.
7. Petri dishes.
8. 50 mL Conical centrifugation tubes.
9. 1.5 mL Microcentrifuge tubes.
10. Aerosol-resistant tips for micropipettes (20, 200, and 1000 μ L tips).
11. 5, 10, and 25 mL pipettes.
12. Glass beads.
13. Sonicator.
14. Commercial blender (7011HS, Waring).
15. Skin punch biopsy tool, sterile and disposable (Acuderm, Inc.).
16. Tissue homogenizer Pro 200 Series.
17. Bacterial incubator overnight.
18. ChemiDoc XRS+ (Bio-Rad).

3 Methods

3.1 Preparing the Inoculum of a Mid-Logarithmic Bioluminescent *S. aureus* Strain

1. Streak bioluminescent *S. aureus* strain onto blood agar plates (TSA with 5% sheep blood).
2. Grow the colonies on the plates by incubating them at 37 °C for approximately 16 h (overnight).
3. Select at least three individual bacterial colonies and culture in TSB (37 °C and shaking at 240 rpm) for approximately 16 h (overnight).
4. Perform a subculture with 1:50 dilution of the overnight culture to obtain mid-logarithmic growth-phase bacteria (approximately 2-h duration).

5. Pellet, resuspend, and wash the bacteria 2–3 times in sterile PBS.
6. Pellet and resuspend the bacterial subculture inoculum to a predetermined inoculum by determining the optical density absorbance at 600 nm. The typical inocula for each of the various models are listed below:
 - a. Intradermal skin infection mouse model: 1×10^6 – 1×10^8 CFU/100 μ L PBS.
 - b. Epicutaneous skin inflammation mouse model: 1×10^8 CFU/100 μ L PBS.
 - c. Incisional wound infection mouse model: 1×10^8 CFU/10 μ L PBS.
 - d. Excisional wound infection mouse model: 1×10^7 CFU/100 μ L PBS.
 - e. Orthopedic implant infection mouse model: 5×10^2 – 1×10^3 CFU/2 μ L PBS.
 - f. Hematogenous orthopedic implant infection mouse model: 1×10^7 CFU/100 μ L PBS.
 - g. Orthopedic implant infection rabbit model: 1×10^4 CFU in 10 μ L PBS.
7. Pipette 1 mL of sample into a 12 $\times$ 75 mm polystyrene tube. Verify and record the luminescence of 1 mL sample using the luminometer.
8. Verify the CFU in the inoculum by culturing the bacteria overnight on TSA plates.

3.2 Procedures of Intradermal Skin Infection

1. Anesthetize wt C57BL/6 mice, pIL1-DsRed reporter mice, or LysM-EGFP mice (2% isoflurane), and shave the dorsal back with clippers.
2. With the lower back skin taut and syringe bevel facing up, intradermally (i.d.) inject the desired inoculum of bioluminescent *S. aureus* in 100 μ L of PBS using a 29-gauge insulin syringe (*see* Note 2).
3. Prior to in vivo imaging (*see* Subheading 3.9, below), digital photographs can be taken of the mouse skin.

3.3 Procedures of Epicutaneous Skin Inflammation

1. Anesthetize mice (e.g., 2% isoflurane), and carefully shave the dorsal skin with clippers. Ensure that the entire dorsal back, from the base of the neck to the lower flanks, as well as the sides are shaved to ease future bandage application and removal (*see* Note 3).
2. Depilate the shaved back for 30–60 s with enough depilatory cream (Nair) to cover the shaved area (*see* Note 4).
3. Wipe off hair and excess depilatory cream with water-soaked sterile cotton gauze pads until thoroughly removed, and let mice acquiesce for 24 h (*see* Note 5).

4. Pipette 100 μ L volume of PBS containing 1×10^8 CFU of a bioluminescent *S. aureus* strain onto a sterile gauze pad (1.5×1 cm).
5. Secure the gauze pad to the shaved back with transparent bio-occlusive dressing (Tegaderm; 3 M) ensuring that there are no air entryways, followed by two layers of adhesive bandages (BAND-AID, Johnson and Johnson) for 7 days. Each mouse should be placed with its unshaved ventral side positioned onto the nonadhesive gauze portion of the adhesive bandage. Each of the two adhesive sides of the bandage should then be tightly wrapped around the back of each mouse and secured on top of the bio-occlusive dressing. The second layer of adhesive bandage should be applied in a similar manner. This ensures maximal comfort for the animal and long-lasting bandage integrity (see Note 6).
6. Check the status of the adhesive bandages every 2 days to ensure that they are still intact as the mice may gnaw at it. If this is the case, add no more than one more bandage per mouse. Excess gnawed-at bandages, particularly around the shoulders and chest of the mouse, may be trimmed to further reduce the likelihood of additional chewing.
7. After 7 days, carefully remove bandages, bio-occlusive dressing, and gauze pad. Then gently scrape the inflamed skin with sterile forceps to remove non-adherent bacteria, and allow the infected area to rest for at least 1 h prior to taking the initial photographs and in vivo BLI imaging.
8. Prior to in vivo imaging (see Subheading 3.9, below), digital photographs can be taken of the mouse skin.

3.4 Procedures of Incisional Wound Infection Mouse Model

1. When using diabetic mice, to mimic type II diabetic conditions, confirm that the mice are hyperglycemic (blood glucose level above 300 mg/dL) before proceeding by making a small incision in the tail vein with a #11 scalpel blade to form a blood droplet, and measure blood glucose with test strip and glucometer.
2. Anesthetize mice with 2% isoflurane, and shave the back skin with clippers.
3. Perform three parallel 8 mm full-thickness cuts into subcutaneous fat with ~1.5 mm space between each cut on the upper dorsal back skin, using a #11 scalpel blade (see Note 7).
4. Pipette a total of 1×10^8 CFU *S. aureus* in 10 μ L PBS distributed evenly into the three scalpel cuts.
5. Prior to in vivo imaging (see Subheading 3.9, below), digital photographs can be taken of the mouse skin.

3.5 Procedures of Excisional Wound Infection Mouse Model

1. Anesthetize mice with 2–3% isoflurane, and shave the back skin with clippers.
2. Prepare the shaved region with 10% w/v povidone-iodine and 70% ethanol.

3. Perform a full-thickness 6 mm punch biopsy wound into subcutaneous fat just above the dorsal back skeletal muscle layer (Fig. 5).
4. Inject a total of 1×10^7 CFU of *S. aureus* in 100 μ l PBS beneath the fascia and centered in the wound bed.
5. Prior to in vivo imaging (*see* Subheading 3.9, below), digital photographs can be taken of the mouse skin.

3.6 Procedures of Orthopedic Implant Infection Mouse Model

1. Anesthetize mice with 2% inhalation of isoflurane and use vet ointment on eyes to prevent dryness while under anesthesia.
2. Place mice on a hard-surface water-circulating heating pad set to 37 °C.
3. Assess the appropriate level of anesthesia by observing the respiratory rate, muscle tone, toe pinch, corneal reflex, and color of mucous membranes.
4. Cover the mice with a sterile surgical drape with a hole at the surgery site on the right knee.
5. Inject buprenorphine (sustained-release formulation) (2.5 mg/kg) subcutaneously just prior to surgery. Additional doses of sustained-release buprenorphine may be administered at 3-day intervals as needed for analgesia.
6. Shave the operative knee and prep using three alternating scrubs using povidone-iodine prep pads and alcohol prep pads.
7. Perform a midline incision in the skin overlying the right knee joint. Extend the skin incision so that the extensor mechanism is well defined.
8. Perform a medial parapatellar arthrotomy, and sublux the quadriceps-patellar tendon extensor mechanism laterally with an Adson forceps. This brings the intercondylar notch of the femur into plain view.
9. Manually ream the intramedullary canal using a 25-gauge needle (*see* Note 8).
10. Insert a medical-grade titanium K-wire (9 mm length, 0.6 mm diameter) by using a press-fit technique, which entails manually pushing it using a pin holder, in a retrograde direction into the intramedullary canal and keep the end of the K-wire extended approximately 1 mm into the knee joint space.
11. Using a micropipette, pipette 2 μ L of typically 1×10^3 CFU of bioluminescent *S. aureus* onto the tip of the implant within the knee joint space (*see* Note 9).
12. Reduce the quadriceps-patellar complex back to midline using forceps, and close the overlying subcutaneous tissue and skin using absorbable subcuticular sutures.
13. Do not leave an animal unattended until it has regained sufficient consciousness to maintain sternal recumbency. Do not return an animal that has undergone surgery to the company of other animals until fully recovered.

3.7 Procedures of Hematogenous Orthopedic Implant Infection Model

1. These mouse surgical procedures are identical to the procedures described in the orthopedic implant infection mouse model, with the exception that no bacteria are inoculated into the knee joint space in **step 11**, above.
2. At 21 days post-surgery, anesthetize mice via inhalation of isoflurane (2%), and intravenously inject an inoculation of a bioluminescent *S. aureus* strain (typically at 1×10^7 CFU via the retro-orbital vein).

3.8 Procedures of Orthopedic Implant Infection Rabbit Model

1. Anesthetize rabbits via intramuscular (i.m.) injection of a mixture of ketamine (25 mg/kg) and xylazine (1.5 mg/kg), in combination with maintained inhalation of isoflurane (1.5%). Use vet ointment on eyes to prevent dryness while under anesthesia.
2. Place rabbits on a hard-surface water-circulating heating pad set to 37 °C.
3. Assess the appropriate level of anesthesia by observing the respiratory rate, muscle tone, toe pinch, corneal reflex, and color of mucous membranes.
4. Cover the rabbits with a sterile surgical drape with a hole at the surgery site on the right knee.
5. Inject sustained-release buprenorphine (0.2 mg/kg) and sustained-release meloxicam (0.6 mg/dose) subcutaneously (s.c.) for analgesia.
6. Inject metoclopramide (0.3 mg/kg) s.c. as a gastrointestinal pro-motility agent.
7. Shave the distal anterior right mid-thigh through the proximal leg and prep area using three alternating scrubs using betadine and 70% alcohol.
8. With knee in slight flexion, perform a midline incision in the skin overlying the right knee joint. Extend the skin incision so that the extensor mechanism can be well defined (Fig. 8a).
9. Perform a medial parapatellar arthrotomy, and laterally dislocate the patella to expose the trochlea and intercondylar notch.
10. With knee in maximal flexion, utilize a 2 mm drill bit to access the femoral canal just anterior to Blumensaat's line.
11. Utilize a 2.2 mm drill bit to expand the previously drilled hole to allow room for the head of the peg implant to sit flush with the articular surface.
12. Pipette 10 μ L of 1×10^4 CFU of a bioluminescent *S. aureus* strain into the femoral canal.
13. Manually insert the titanium locking peg implant until flush with the articular surface.
14. Relocate the patella, and close the surgical site in layered fashion with interrupted 4-0 Vicryl sutures.

15. Confirm implant placement by anteroposterior and/or lateral X-rays (Fig. 8a).
16. Do not leave an animal unattended until it has regained sufficient consciousness to maintain sternal recumbency. Do not return an animal that has undergone surgery to the company of other animals until fully recovered.

3.9 In Vivo BLI and FLI

1. Perform in vivo BLI using a whole-animal in vivo imaging system, such as Lumina III IVIS with Living Image Software® (PerkinElmer), as described below:
 - a. Select “Luminescent” and confirm the choice of an “Open Filter” selection.
 - Set desired field of view (FOV).
 - Set desired acquisition time (exposure).
 - Set desired binning (digital pixel binning).
 - Set F-stop (aperture).
 - Set subject height (e.g., mouse = 1.5 cm) and focus to “Use Subject Height.”
 - b. Below are the choices that we typically use for each *S. aureus* infection model:
 - Intradermal skin infection mouse model: FOV = C or D, exposure = 1–5 min, binning = medium, and aperture = 1.
 - Epicutaneous skin inflammation mouse model: FOV = C or D, exposure = 1–5 min, binning = medium, and aperture = 1.
 - Incisional wound infection mouse model: FOV = C or D, exposure = 1–5 min, binning = medium, and aperture = 1.
 - Excisional wound infection mouse model: FOV = C or D, exposure = 1 min, binning = small, and aperture = 1.
 - Orthopedic implant infection mouse model: FOV = D, exposure = 5 min, binning = large, and aperture = 1.
 - Hematogenous orthopedic implant infection mouse model: FOV = D, exposure = 5 min, binning = large, and aperture = 1.
 - Orthopedic implant infection rabbit model: FOV = D, exposure = 5 min, binning = large, and aperture = 1.
2. In some models, sequential in vivo FLI imaging was performed after in vivo BLI with fluorescent reporter mice and using a whole-animal in vivo imaging system, such as the Lumina III IVIS with Living Image Software® (PerkinElmer) (e.g., intradermal skin infection mouse model, incisional wound infection mouse

model, excisional wound infection mouse model, and orthopedic implant infection mouse model), as described below:

- a. Select “Fluorescence” and set appropriate excitation and emission wavelengths, and exposure time.
 - For DsRed fluorescence using the pIL1-DsRed reporter mice:
 - Excitation: 535 nm.
 - Emission: 575–650 nm.
 - Exposure time: typically 0.5 s.
 - F/Stop: 2
 - For EGFP fluorescence using the LysM-EGFP reporter mice:
 - Excitation: 465 nm.
 - Emission: 515–575 nm.
 - Exposure time: typically 0.5–1 s.
 - F/Stop: 2
 - Other desired fluorescent excitation and emission options can be preselected or manually chosen for a multitude of fluorophores.
3. Choose the “Autosave To” icon on the top bar to select where to save the images.
4. For all mouse models of *S. aureus* infection, anesthetize mice with inhalation isoflurane (2%) or intraperitoneal (i.p.) injection of ketamine (100 mg/kg) and xylazine (20 mg/kg) mixture and place them dorsal side up into the imaging chamber. In the orthopedic implant infection mouse model and the hematogenous orthopedic implant infection mouse model, the mice are placed ventral side up into the imaging chamber. Mouse noses should be placed in the isoflurane-connected nosecones to maintain anesthesia. Depending on the desired FOV setting, 1–5 (or more) mice can be imaged at a single time. For the orthopedic implant infection rabbit model, anesthetize rabbits via i.m. injection of ketamine (25 mg/kg) and xylazine (1.5 mg/kg) mixture and place semi-upright into the imaging chamber with the postoperative knee almost maximally flexed and centered under the camera.
5. Close and latch door, and then click “Acquire” to capture the in vivo BLI and/or in vivo FLI signals.
6. In most mouse models and the orthopedic implant infection rabbit model, in vivo BLI signals are quantified as total flux (also known as total radiance) (photons/s), and in vivo FLI signals are quantified as total radiant efficiency ([photons/s]/[mW/cm²]) in a region of interest (ROI) by first expanding the “ROI Tools” section of the “Tool Palette.” In the orthopedic implant infection mouse model and the hematogenous orthopedic implant infection mouse model, in vivo

BLI signals are typically quantified as maximum flux (photons/s/cm²/steradian) within the same ROI.

7. Select the “Circle Icon” and the number of region of interests (ROIs) that correspond to the number of subject animals in the FOV. Resize the ROIs to encompass the desired bioluminescent signals to be quantified.
8. Select “Measure ROIs” in “ROI Tools” in the “Tool Palette,” and the “ROI Measurement Window” will appear. Total flux (photons/sec) is the sum of all of the bioluminescent pixel signals within the ROI. Maximum flux (photons/s/cm²/steradian) is the maximal bioluminescent pixel signal within the ROI. Total radiant efficiency ([photons/s]/[μW/cm²]) is the sum of all of the fluorescent pixel signals within the ROI.
9. Choose the “SELECT ALL” and “COPY” tabs in the bottom right-hand corner of this window to transfer the information to the clipboard and allow pasting into subsequent programs for analysis.

3.10 Procedures of Ex Vivo CFU Enumeration

3.10.1 Procedures for harvesting skin, joint tissue, and organ samples and implants

1. Begin harvests after appropriately euthanizing the animals.
2. For harvesting skin in the intradermal infection model, incisional wound infection model, and excisional wound infection model:
 - a. Use a skin punch biopsy tool (typically use 10 mm diameter) to cut lesional skin, and then use sterile forceps to place each specimen in a 2 mL cryovial. Weigh each specimen so that the CFU/mg tissue can be calculated. Add 500 μL of sterile PBS.
 - b. Keep samples on ice or in fridge until ready to plate.
3. For harvesting infected skin of the epicutaneous skin inflammation model:
 - a. Use sterile surgical scissors to cut a 2 × 2 cm area of skin that showed BLI signals, and then use sterile forceps to place each specimen in a 2 mL cryovial. Weigh each specimen so that CFU/mg tissue can be calculated. Add 500 μL of sterile PBS.
 - b. Keep samples on ice or in fridge until ready to plate.
4. For harvesting infected joint/bone tissue and implants in the orthopedic implant infection mouse model and the hematogenous orthopedic implant infection mouse model.
 - a. Sterilize the skin surface overlying knee joint with 70% ethanol. Allow to dry.
 - b. Make an incision at the knee joint, part the skin to expose the joint space, and be careful not to cut out the infected tissue.

- c.** Cut above and below the knee joint. Be sure to take out all infected tissue and bone.
 - d.** Crush the excised bone and remove the implant from the proximal end of the femur to avoid contamination from the infected bone/soft tissue.
 - e.** To solubilize bacteria from the K-wire implants, place each implant into 1 mL TSB with 0.3% Tween 20, vortex for 2 min, sonicate for 10 min, and vortex again for 2 min.
 - f.** Place bone and joint tissue in a 2 mL cryovial with 500 μ L sterile PBS and place on ice (*see Note 10*).
 - g.** Keep samples on ice or in fridge until ready to plate.
- 5.** For harvesting and homogenizing infected joint and bone tissue and sonicating implants in the orthopedic implant infection rabbit model:
 - a.** Sterilize the skin surface overlying knee joint with 70% ethanol. Allow to dry.
 - b.** Make an incision at the knee joint, part the skin to expose the joint space, and be careful not to cut out the infected muscle and tendon tissue.
 - c.** Cut out all muscle and tendon tissue from femur.
 - d.** Crush the femur manually and remove the implant from the femur to avoid contamination from the infected tissue.
 - e.** Place muscle and tendon tissue in a blender container containing 200 mL of PBS (*see Note 10*).
 - f.** Place crushed femur bone specimens in a blender container containing 50 mL of PBS (*see Note 10*).
 - g.** Separately homogenize muscle/tendon specimens and bone specimens at 20,000 rpm ($45,000 \times g$) for 30–120 s in a commercial blender.
 - h.** To solubilize bacteria from the peg implants, place each implant specimen in a conical tube with 2 mL TSB with 0.3% Tween 20, vortex for 2 min, sonicate for 10 min, and vortex again for 2 min.
 - i.** Keep samples on ice or in fridge until ready to plate.
- 6.** For harvesting organs from any of the mouse models:
 - a.** Sterilize the skin surface of entire ventral side of the mouse.
 - b.** Make a midline excision and collect organ specimens through careful dissection.
 - c.** Place each organ specimen in a 2 mL cryovial with 500 μ L sterile PBS (*see Note 10*).
 - d.** Keep samples on ice or in fridge until ready to plate.

3.10.2 Procedures for Homogenizing Skin, Joint Tissue, and Organ Specimens from Mice (for Homogenizing Specimens from Rabbits, Please See Subheading 3.10.1, step 5, above)

1. Perform all work inside a biosafety cabinet as homogenization can create aerosols. Homogenizer should only be turned on while submerged to prevent aerosols and splashes.
2. Set up a rack on ice that holds four 50 mL conical centrifugation tubes for rinsing the homogenizer between specimens. Set up the order of tubes so that tubes 1 and 2 contain 40 mL of 70% ethanol and tubes 3 and 4 contain 40 mL of sterile PBS.
3. Homogenize samples in each 2 mL cryovial with 500 μ L sterile PBS by placing the homogenizer tip into the cryovial while on ice. Turn on the homogenizer by starting on the lowest speed and working up to the highest speed. Homogenize to a uniform consistency by varying the speed from high to low until sample becomes uniform and all the tissue (and bone) pieces become a homogenous slurry (*see Note 11*).
4. Rinse the homogenizer between samples by sequentially submerging and turning on the homogenizer in tubes 1 through 4.
5. Wipe the homogenizer tip clean with a fresh kim-wipe after rinsing.
6. Repeat **steps 4–5** for each sample.
7. Add 500 μ L sterile PBS to each tissue and organ specimen to bring the total volume to 1000 μ L.

3.10.3 Procedures for Plating Serial Dilutions, Culturing, and Enumerating CFU for all Models

1. Make serial dilution tubes containing 900 μ L of sterile PBS to make tenfold dilutions of the homogenate (e.g., $-10\times$, $-100\times$, $-1000\times$, $-10,000\times$, $-100,000\times$)
2. Vortex each homogenized specimens for 10 s.
3. Pipet 100 μ L of homogenate into first tube. Vortex this tube for 10 s, then pipet 100 μ L into the next serial dilution tube, and repeat the serial dilutions so that the appropriate number of CFU can be counted (*see Note 12*).
4. Put 5–10 sterile beads into each TSA agar plate.
5. Vortex each sample for 10 s immediately prior to plating.
6. Pipet 100 μ L of each serial dilution onto the TSA plates containing the sterile beads.
7. Put plate lid back on and shake back and forth while maintaining the plate horizontal. Try to get an even coat of the solution onto the TSA surface.
8. Dispose of beads from the plates into 10% bleach solution.

9. Repeat **steps 1–8** for all samples.
10. Culture the plates in a 37 °C bacterial incubator overnight.
11. Count CFU. This can be done manually. Alternatively, CFU counting can be done automatically using the ChemiDoc XRS + with Image Lab 4.1 to take the photographic image of the plate and the CFU can be enumerated using the software program Quantity One (*see* Note 13).

4 Notes

1. Female mice are typically not used because the size of their femurs is too small for the surgical procedures in this model.
2. A successful i.d. injection will correspond with the formation of a white-appearing indurated bulge in the mouse skin and a peak in vivo BLI signal on day 1 postinfection (Fig. 2b). If these do not occur, the bacteria were likely injected too deeply (i.e., subcutaneously).
3. Accidentally nicking the skin with the clippers will induce local skin inflammation and change the outcome of the results. Should this occur, do not use the mouse for the experiment.
4. Excess timing of depilatory cream application will cause skin inflammation and negatively affect the experiment.
5. Failure to remove the depilatory cream will cause skin inflammation. Should this occur, the mice should not be used for the experiment.
6. Tight contact between the bacteria-soaked gauze pad and the skin is extremely important for consistent skin infection. However, be careful not to wrap the bandage too tight, as this can make it difficult for the mice to breathe and could lead to suffocation. Carefully monitor the mice after the procedure to make sure that they are breathing properly and adjust the bandages accordingly.
7. Use forefinger and thumb to stretch the skin so it is taut. This allows the scalpel blade to make a smooth incision.
8. Care must be taken to remain parallel to the femoral shaft to avoid asymmetric reaming and potential femur fracture.
9. Use of an inoculation volume of more than 2 μ L leads to wider tissue contamination and less discrete imaging.
10. Weigh each specimen so that the CFU/mg tissue can be calculated.
11. Be careful not to keep the homogenizer on the high setting as the sample can overheat and this can kill the bacteria.
12. The homogenate may be very viscous so the use of wide-opening pipet tips is advised. Alternatively, the end of the pipet tip may be cut with sterile scissors to create a wider opening.

13. Avoid automated counting of plates with >2000 CFUs because the numbers are no longer accurate.

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Fig. 1.

Representative schematic of an in vivo imaging system (IVIS). Representative schematic of a typical in vivo optical imaging system (IVIS Spectrum [PerkinElmer]) consisting of a light-tight (dark) chamber with an adjustable sample stage that is maintained at 37 °C (e.g., for placement of anesthetized animals). The imaging system is equipped with an LED light source to obtain a reference grayscale photograph of the subject; an excitation emission light source; a filter wheel to detect specific wavelengths of light; and a highly sensitive cooled integrating charge-coupled device (CCD) camera to detect photons of lights from in vivo BLI and in vivo FLI signals generated from the subject

Fig. 2.

In vivo BLI and in vivo FLI of a *S. aureus* intradermal (i.d.) infection model in mice. C57BL/6 wild-type mice, pIL1-DsRed reporter mice, or LysM-EGFP mice were inoculated i.d. with *S. aureus*. (a) Representative skin lesions (left) (entire dorsal backs [top panels, millimeter ruler shown for scale] and close-ups of lesions [bottom panels]) and mean total lesion size (cm²) ± standard error of the mean (SEM) (right). (b) Representative in vivo BLI signals (left) and mean total flux (photons/s) ± SEM (logarithmic scale) (right). (c) Representative in vivo EGFP-neutrophil FLI signals (left) and mean total radiant efficiency (photons/s)/(mW/cm²) ± SEM (right). (d) Representative in vivo DsRed-IL-1β FLI signals (left) and mean total radiant efficiency (photons/s)/(mW/cm²) ± SEM (right). **P* < 0.05, † *P*

< 0.01 , *S. aureus* -infected mice versus none (sham injection of sterile PBS) (two-tailed Student's *t*-test) (Original figure from [12])

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Fig. 3. In vivo BLI in a *S. aureus* epicutaneous inflammation mouse model. (a) Timeline of the *S. aureus* epicutaneous model. (b) Representative photographs of the mouse back skin (left, top panels) and in vivo BLI signals (left, bottom panels) of C57BL/6 mice on day 7 after epicutaneous *S. aureus* or PBS (control) exposure and quantified in vivo BLI signals as total flux (photons/s) $\pm$ standard error of the mean (SEM) (right). The limit of detection is 2×10^4 photons/s. (c) Representative bacterial culture plates after overnight culture of skin homogenates from mice after epicutaneous exposure with *S. aureus* or PBS (left) and quantified as ex vivo CFU $\pm$ SD (right). * $P < 0.01$, ‡ $P < 0.001$, *S. aureus*-infected mice versus PBS (epicutaneous exposure of sterile PBS without any bacteria) (two-tailed Student's *t*-test)

Fig. 4.

Diabetic mouse model of CA-MRSA wound infection. Three parallel scalpel wounds 8 mm in length and ~1.5 mm apart were inoculated with 1×10^8 CFU/10 μ L of bioluminescent strain SAP231 (parent strain NRS384) or no bacteria. **(a)** Representative photographs of the wounds (top panels) with close-ups (bottom panels). **(b)** Total wound size (cm²) $\pm$ SEM. **(c)** Representative *in vivo* BLI signals on a color scale overlaid on a grayscale photograph of the mice. **(d)** Mean total flux (photons/s) $\pm$ SEM (logarithmic scale)

Fig. 5.

In vivo BLI and FLI in an excisional wound *S. aureus* infection model. LysM-EGFP mice were administered a full-thickness 6 mm wound on the dorsum and infected with 1×10^7 CFU bioluminescent *S. aureus* and imaged in an IVIS Spectrum. (a) Representative wound healing photographs and (b) mean wound size (cm²) $\pm$ standard error of the mean (SEM). (c) Representative in vivo BLI signals and (d) mean total flux (photons/s) $\pm$ SEM (logarithmic scale). (e) Representative in vivo FLI signals and (f) mean total radiant efficiency (photons/s/cm²/steradian) $\pm$ SEM. $N = 4$ mice per group. * $P < 0.05$, *S. aureus*-infected mice versus -uninfected mice (sham injection of sterile PBS) (two-tailed Student's *t*-test)

Fig. 6.

In vivo BLI and *in vivo* FLI in an orthopedic implant infection mouse model. After surgical placement of an orthopedic-grade stainless steel K-wire into the distal femur, 5×10^2 CFUs/2 μ l of *S. aureus* or 2 μ l of saline alone (uninfected) was inoculated into the knee joint in the area of the cut end of the implant. **(a)** *In vivo S. aureus* BLI as measured by mean maximum flux (photons/s/cm²/steradian) $\pm$ SEM. **(b)** Representative *in vivo* BLI signals on a color scale overlaid on top of a grayscale image of mice. **(c)** *In vivo* FLI of LysM-EGFP mice (EGFP-neutrophil infiltration), as measured by total radiant efficiency (photons/s)/(mW/cm²) $\pm$ SEM. **(d)** Representative *in vivo* FLI signals of LysM-EGFP mice on a color scale overlaid on top of a grayscale image of mice. * $P < 0.05$, † $P < 0.01$, ‡ $P < 0.001$, *S. aureus*-infected mice versus none (sham injection of sterile PBS) (two-tailed Student's *t*-test) (Original figure from [14])

Fig. 7.

Model of hematogenous implant infection. **(a)** Timeline of hematogenous infection of an orthopedic implant. **(b)** Representative *in vivo* BLI signals from internal organs and the postsurgical leg possessing a surgically implanted implant on a color scale overlaid on a grayscale photograph of mice following an intravenous inoculation with a bioluminescent *S. aureus* strain (1×10^7 CFU). **(c)** Representative images of *ex vivo* BLI signal from dissected organs and legs of the mice, harvested on day 28 after the intravenous *S. aureus* inoculation

A Surgical procedures

B Representative *in vivo* BLI (photons/second)

Fig. 8.

Rabbit orthopedic infection model. (a) Right leg was prepped for surgery followed by a medial parapatellar arthrotomy. The patella was laterally dislocated to expose the intercondylar notch, anterior to the intercondylar notch the femoral intramedullary canal was drilled, countersunk, and *S. aureus* (bioluminescent strain SAP231, 1×10^4 CFU in 10 μ L PBS) was pipetted into the canal. A surgical peg implant was inserted into the canal manually with a screwdriver until the implant peg was flush with the articular surface. The surgical site was closed by relocating the patella and using interrupted absorbable sutures in a layered fashion. Lateral (or anteroposterior) X-rays confirm implant placement. (b) Representative *in vivo* BLI signals overlaid on a grayscale photograph of the postoperative rabbit knees. Color scale bar shown is luminescence (minimum 5×10^4 and maximum 2×10^6 radiance [photons/s/cm²/steradian])