Optical imaging for bacterial infections: skin, joint and bone

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Methicillin-resistant Staphylococcus aureus (MRSA)

The grapes of wrath

Staphylococcus aureus and MRSA skin infections

- *S. aureus* skin infections result in 12 million outpatient and emergency room visits and 500,000 hospital admissions per year in the U.S.

- MRSA is the leading cause of skin infections presenting to emergency rooms in the U.S.

Staphylococcus aureus and MRSA

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A hallmark of *S. aureus* infections is the neutrophilic abscess
1. Intradermal infection model
Intradermal inoculation of bioluminescent S. aureus (2x10^6 CFU/100μl)

Bioluminescent S. aureus, pIL-1β-dsRed and Lys-EGFP reporter mouse

(in vivo bioluminescence and fluorescence imaging) (Xenogen IVIS®)

Lesion sizes (cm²)

Histology, immunohistochemistry and myeloperoxidase (MPO) assays

Levels of cytokine and chemokine gene expression from homogenized skin specimens (QPCR, ELISA, protein arrays)

Mouse model of skin infection with S. aureus
To determine the cell types that produce IL-1β in host defense against *S. aureus* skin infections.
Temporal kinetics of IL-1β production and neutrophil recruitment

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cho, js...miller, ls, et al. 2012. ploS pathogens. 8(11): e1003047
Temporal kinetics of IL-1β production and neutrophil recruitment

Temporal kinetics of IL-1β production and neutrophil recruitment

Few monocytes/macrophages (MOMA2\(^+\) cells) co-localize with IL-1\(\beta\)-producing cells during *S. aureus* skin infection.
Neutrophils (7/4⁺) are the predominant cell type that produces IL-1β at early time points after *S. aureus* skin infection.
Neutrophil-derived IL-1β is sufficient for bacterial clearance of a S. aureus skin infection
Neutrophil-derived IL-1β is sufficient for abscess formation and neutrophil recruitment during a S. aureus skin infection.
2. Infected Wound Model
Mouse model of superficial skin infection with *S. aureus*

Inoculation of bioluminescent CA-MRSA strain (USA300 LAC::lux) into 3 superficial scalpel cuts on the backs of mice

Bacterial counts (*in vivo* bioluminescence) (Xenogen IVIS®)

Lesion sizes (cm²)

Skin lesions of our superficial S. aureus skin infection model.

*In vivo* bioluminescence imaging to measure in the *S. aureus* bacterial burden in real-time.

*In vivo* bioluminescence highly correlated with bacterial CFUs harvested from the infected skin lesions.
Subcutaneous antibiotic treatment of a CA-MRSA wound infection in diabetic mice (NONcNZO10/LtJ) with vancomycin, daptomycin and linezolid

A Total lesion size (cm²)

B in vivo bioluminescence (log scale)

3. Prosthetic joint infection model
Common causes of prosthetic-knee and prosthetic-hip infection

- Gram-positive cocci (approximately 65%)
  - Coagulase-negative staphylococci
  - *Staphylococcus aureus*
  - Streptococcus species
  - Enterococcus species
- Aerobic gram-negative bacilli (approximately 6%)
  - Enterobacteriaceae
  - *Pseudomonas aeruginosa*
- Anaerobes (approximately 4%)
  - Propionibacterium species
  - Peptostreptococcus species
  - *Finegoldia magna*
- Polymicrobial (approximately 20%)
- Culture-negative (approximately 7%)
- Fungi (approximately 1%)
Total Arthroplasties Performed and Prosthetic Infections

Figure 1. Scanning Electron Micrograph of a *Staphylococcus epidermidis* Biofilm on Foreign Material.

Bacteria grow in multicellular clusters. The scale bar represents 10 μm. (Photograph courtesy of Robin Patel, Mayo Clinic College of Medicine.)

Radiolucent lines seen on x-ray represents periprosthetic osteolysis, which is a hallmark of post-arthroplasty infections that lead to implant failure.
Mouse model of post-arthroplasty joint infection with *S. aureus*
Surgical procedures of a mouse model of post-arthroplasty joint infection with S. aureus

In vivo imaging to measure bacterial burden and neutrophil influx in real-time

(1) S. aureus strain is bioluminescent (emits light)

(2) Mice are LysEGFP that possess fluorescent neutrophils

In vivo imaging system was used to detect:

(1) bioluminescence (bacterial burden)

(2) fluorescence (neutrophil signal)

in the infected joints of live anesthetized mice.

Representative colonies of the bioluminescent S. aureus strain on a bacterial culture plate
Other endpoints

Biofilm Formation (VP-SEM)

Bacterial CFUs adherent to the implants and within the infected joint tissue

Neutrophil influx (histology and myeloperoxidase (MPO) assays)

Effects of infection on periprosthetic osteolysis (micro-CT imaging)
The level of inflammation in the post-operative joints can be measured in real-time by using *in vivo* fluorescence imaging of EGFP neutrophil infiltration.

Bernthal, NM... Miller, LS. 2010. *PLOS ONE* 5(9): e12580
Neutrophils admixed with gram-positive bacteria can be detected in the joint tissue surrounding the implant after *S. aureus* infection

Berenthal, NM… Miller, LS. 2010. *PLOS ONE* 5(9): e12580
Biofilm formation was readily observed on the metallic implants after post-operative *S. aureus* infection.

Bernthal, NM... Miller, LS. 2010. *PLOS ONE* 5(9): e12580
Monitoring differences in individual animals

A. *in vivo* Bioluminescence (log scale)

B. *in vivo* EGFP-neutrophil fluorescence (total radiant efficiency)

C. Mouse #1

Mouse #2

Mouse #3

What is the optimal \textit{S. aureus} bioluminescence strain?
What is the optimal S. aureus bioluminescence strain?

Optimal antibiotic therapy
What is the optimal antibiotic therapy against orthopaedic implant infections?

Niska, JA... Miller, LS. 2013. *Antimicrobial Agents & Chemotherapy* 57(10): 5080-5086
What is the optimal antibiotic therapy against orthopaedic implant infections?

A Bacterial CFU harvested from the peri-implant tissue (log scale)

B Bacterial CFU harvested from the implants (log scale)

C Bacterial CFU present in peri-implant tissue after 48 hr culture

D Bacterial CFU present from implants after 48 hr culture

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<th>p-value</th>
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Niska, JA... Miller, LS. 2013. *Antimicrobial Agents & Chemotherapy* 57(10): 5080-5086
What is the optimal antibiotic therapy against orthopaedic implant infections?

A  Representative X-ray images (anteroposterior view)

sham  vanco  vanco + rifampin  uninfected

Niska, JA… Miller, LS. 2013. Antimicrobial Agents & Chemotherapy 57(10): 5080-5086
Bone Implant Interface
(periprosthetic ostelolysis)
Radiolucent lines seen on x-ray represents periprosthetic osteolysis, which is a hallmark of post-arthroplasty infections that leads to implant failure.

Lumina XR
C  S. aureus-infected

Biolum

EGFP

2  5  14  19  28  48  Days

Uninfected

Biolum

EGFP

2  5  14  19  28  48  Days

D  Close-up of day 48 x-ray images

S. aureus-infected  Uninfected

Niska, JA... Miller, LS. 2012. PLOS ONE 7(10): e47397.
Spectrum

Quantum FX micro-CT
Bioluminescence and micro-CT overlay to study the bone-implant interface during infection
Micro-CT images of the mouse femur post-surgery in the presence and absence of S. aureus infection

Niska, JA... Miller, LS. 2012. PLOS ONE 7(10): e47397.
Micro-CT images of the mouse femur post-surgery in the presence and absence of *S. aureus* infection

**A** *S. aureus*-infected

B Uninfected

Histology at day 48 in the presence and absence of S. aureus infection
Support

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Leading research to understand, treat, and prevent infectious, immunologic, and allergic diseases.

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