

A Longitudinal Model of Implant Infection: From Bedside to Bench, and Back Again

Nicholas M. Bernthal, Jonathan R. Pribaz, Alexandra I. Stavrakis, Fabrizio Billi, John S. Cho, Romela I Ramos, Kevin P Francis, Yoichiro Iwakura, and Lloyd S. Miller



Background & Clinical Significance

- **Approximately 10 million metallic implants will be implanted in patients by orthopaedic surgeons annually in the US by 2030.**
- **Implant infection is thought to occur in approximate 1% of all cases**
 - **The most common infecting organism is *Staphylococcus aureus***
- **Implant infections are clinically devastating, leading to reoperations, prolonged antibiotic therapy, extended disability/rehabilitation and significantly worse outcomes.**
- **Costs related to a implant infection sum to an annual national healthcare burden of more than \$12 billion by 2015.**
- **Previous animal studies of implant infection have been in large animals and require large numbers of animals for histological data at different time points**

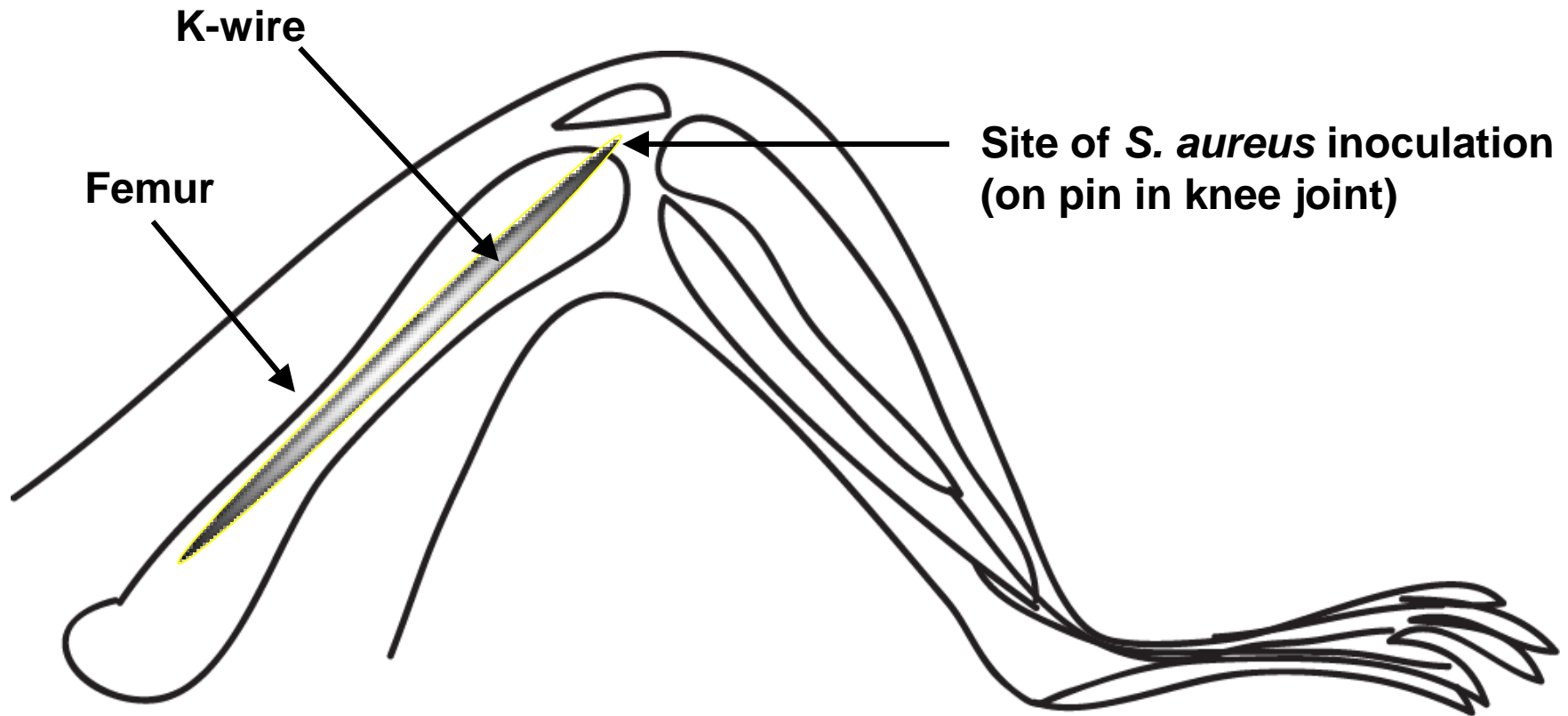
Kurtz,S., et al. 2007. Projections of primary and revision hip and knee arthroplasty in the United States from 2005 to 2030. *J.Bone Joint Surg.Am.* 89:780-785.

2009

A Mouse Model of Post-Arthroplasty *Staphylococcus aureus* Joint Infection to Evaluate *In Vivo* the Efficacy of Antimicrobial Implant Coatings

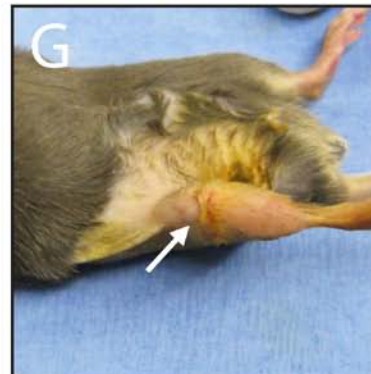
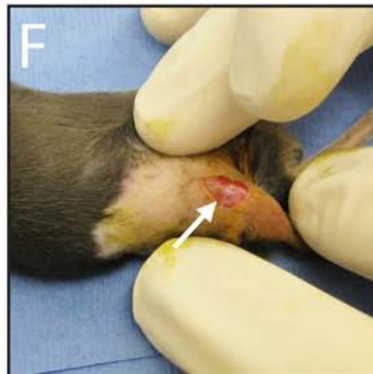
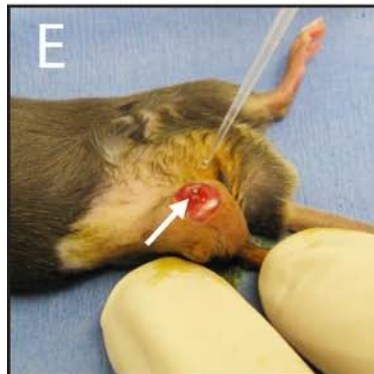
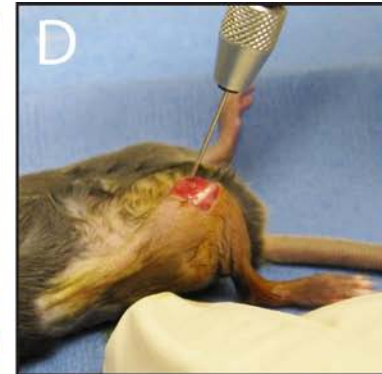
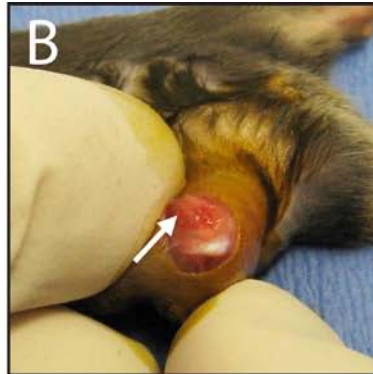
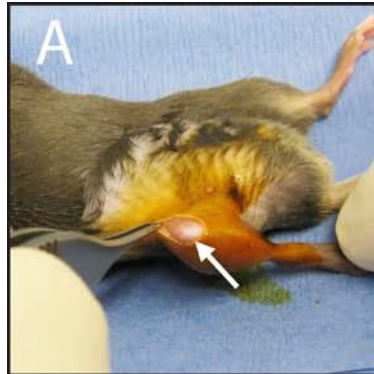
Nicholas M. Bernthal¹, Alexandra I. Stavrakis¹, Fabrizio Billi¹, John S. Cho², Thomas J. Kremen¹, Scott I. Simon³, Ambrose L. Cheung⁴, Gerald A. Finerman¹, Jay R. Lieberman⁵, John S. Adams¹, Lloyd S. Miller^{1,2*}

Mouse model of post-arthroplasty joint infection with *S. aureus*



Surgical technique for placement of an intramedullary K-wire was modified from: Epstein, N.J., et al. 2005. *J. Orthop. Res.* 23:501-510.

Surgical procedures of a mouse model of post-arthroplasty joint infection with *S. aureus*

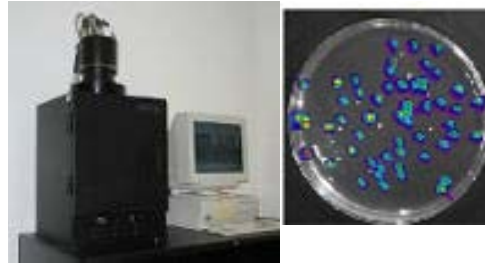


Post-Operative Implant Positioning

QuickTime™ and a
YUV420 codec decompressor
are needed to see this picture.

Mouse model of post-arthroplasty infection with *S. aureus*

Intra-articular inoculation of bioluminescent *S. aureus*



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

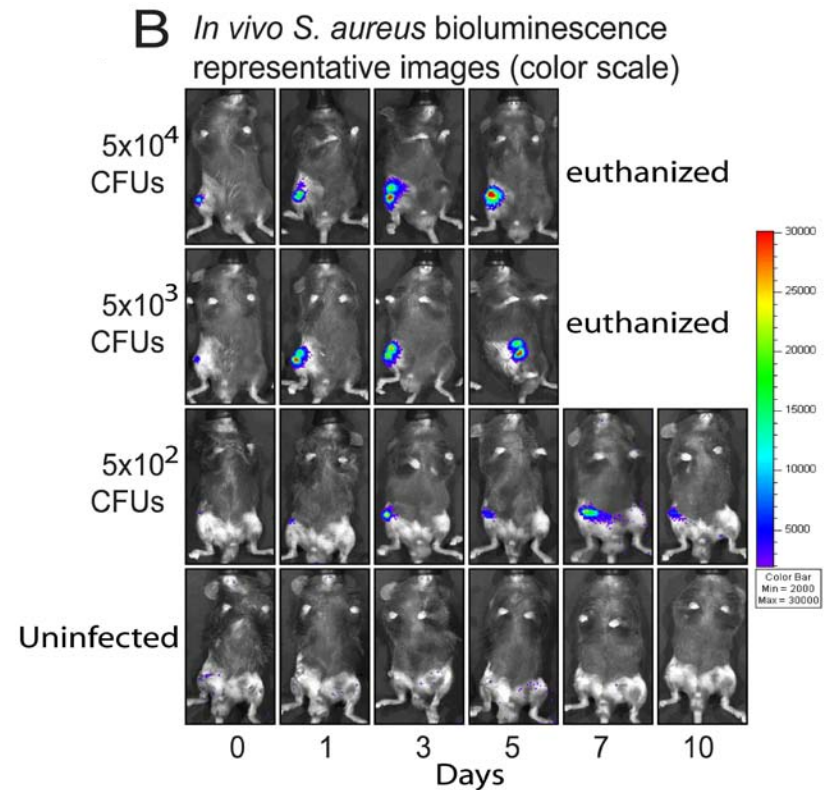
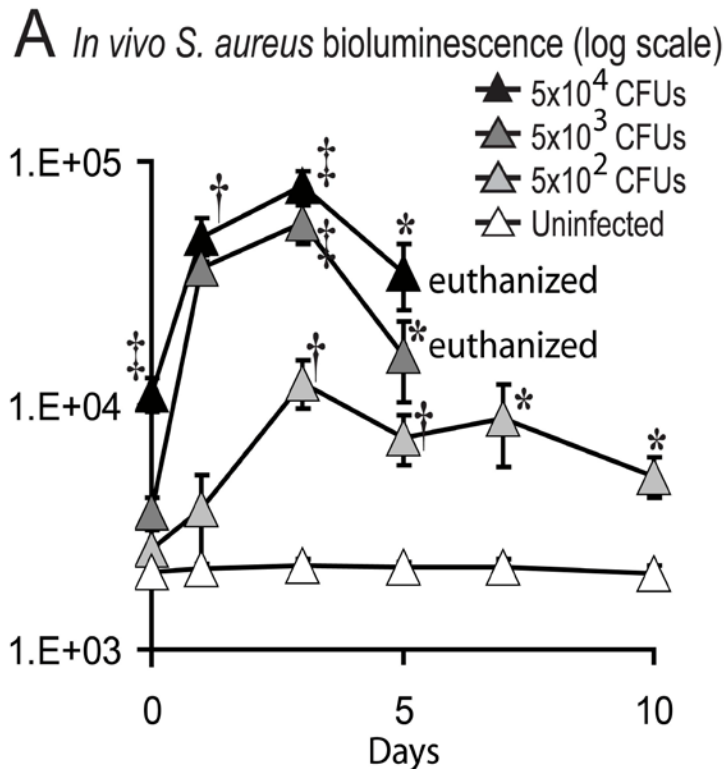


**Histology,
immunohistochemistry
and myeloperoxidase
(MPO) assays**



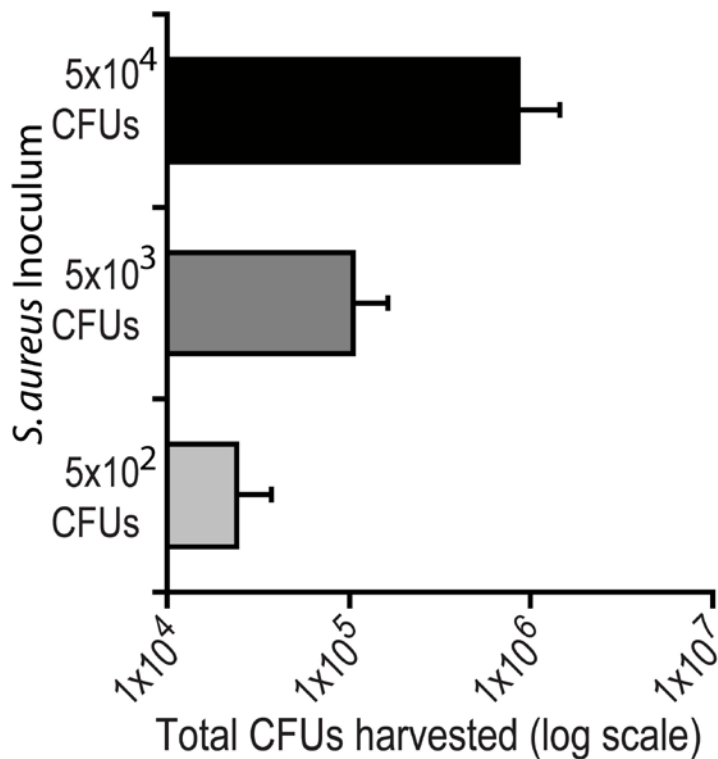
**Variable Pressure
Scanning Electron
Microscopy (VP-SEM)**

The bacterial burden in the post-operative joints can be measured in real-time by using *in vivo* bioluminescence imaging.

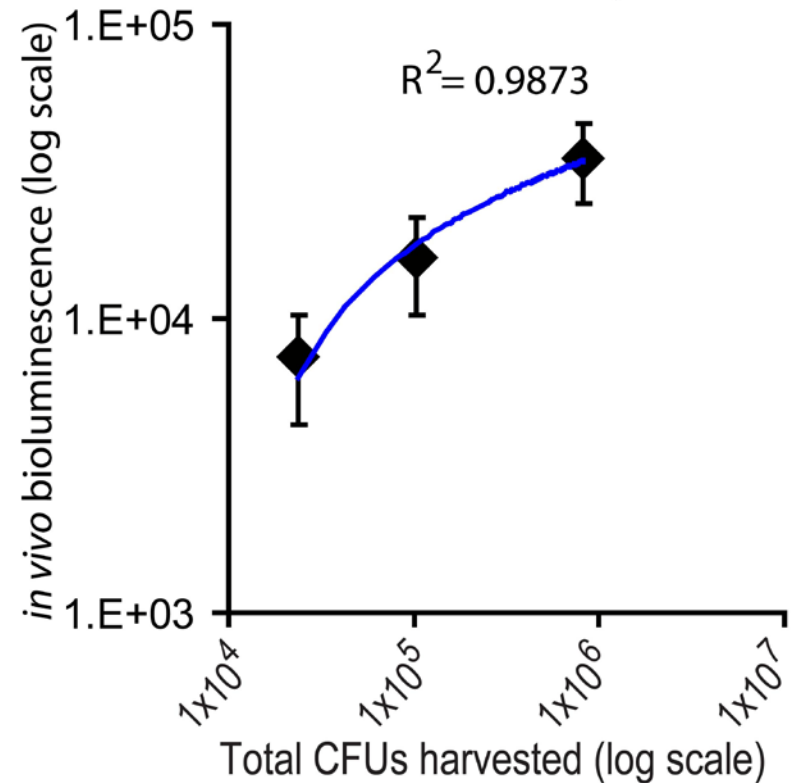


The bacterial burden in the post-operative joints highly correlates with *in vivo* bioluminescence imaging.

Total *S. aureus* CFUs harvested from the implant and joint tissue on Day 5

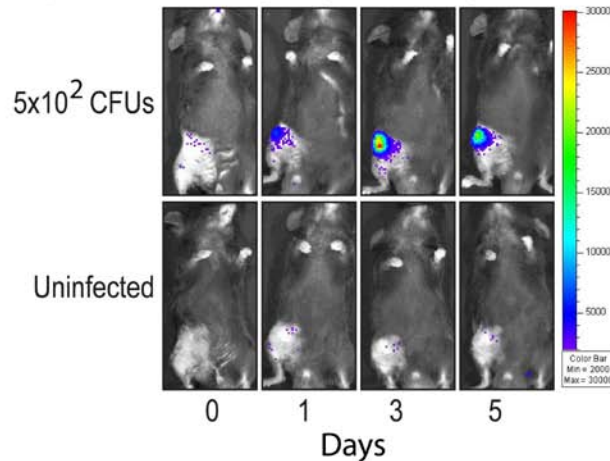


Correlation of *in vivo* bioluminescence and total CFUs harvested on Day 5

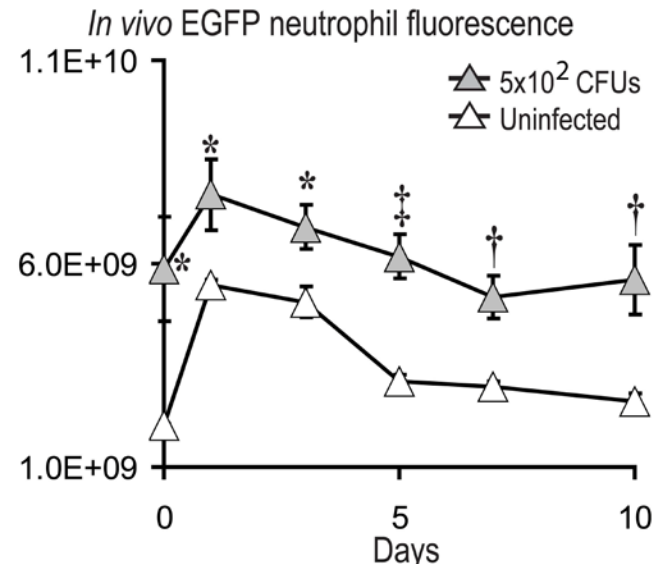
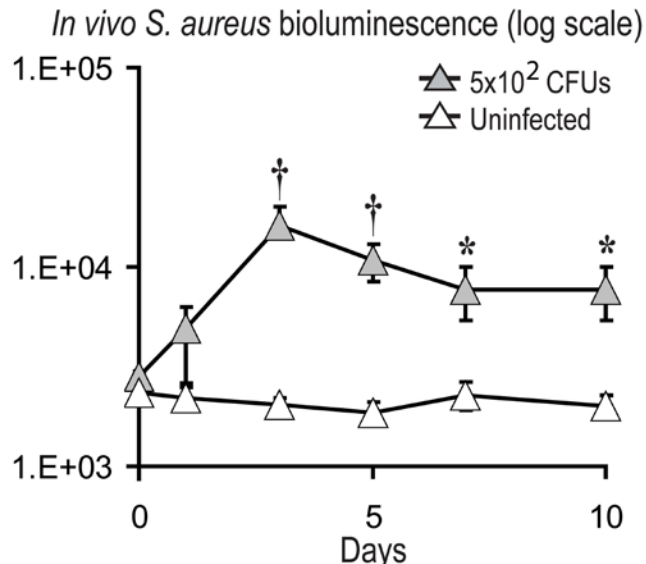
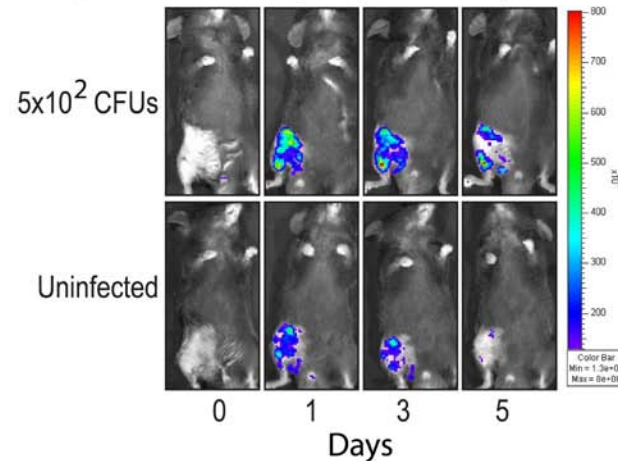


The level of inflammation in the post-operative joints can be measured in real-time by using *in vivo* fluorescence imaging of EGFP neutrophil infiltration

In vivo *S. aureus* bioluminescence representative images (color scale)

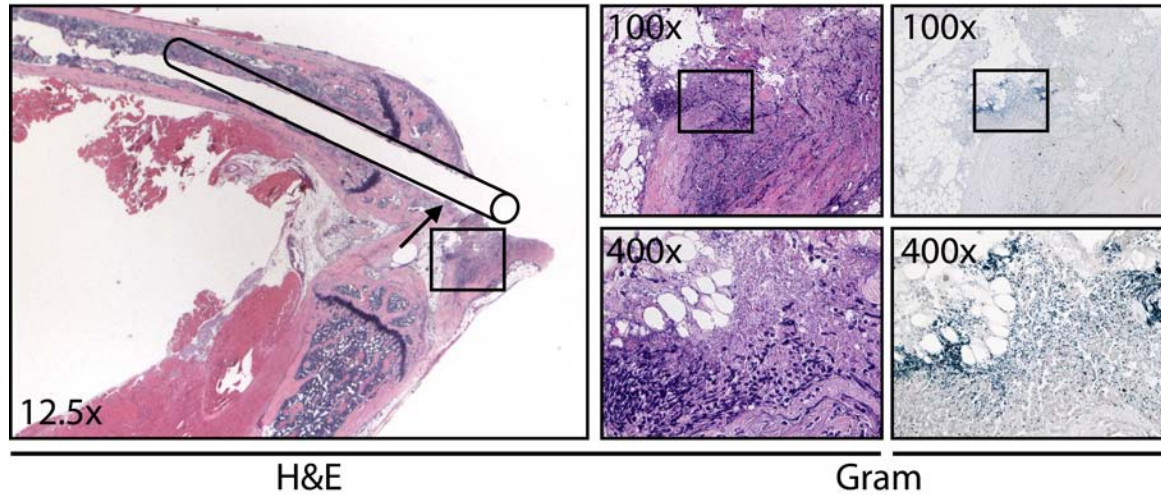


In vivo EGFP neutrophil fluorescence representative images (color scale)

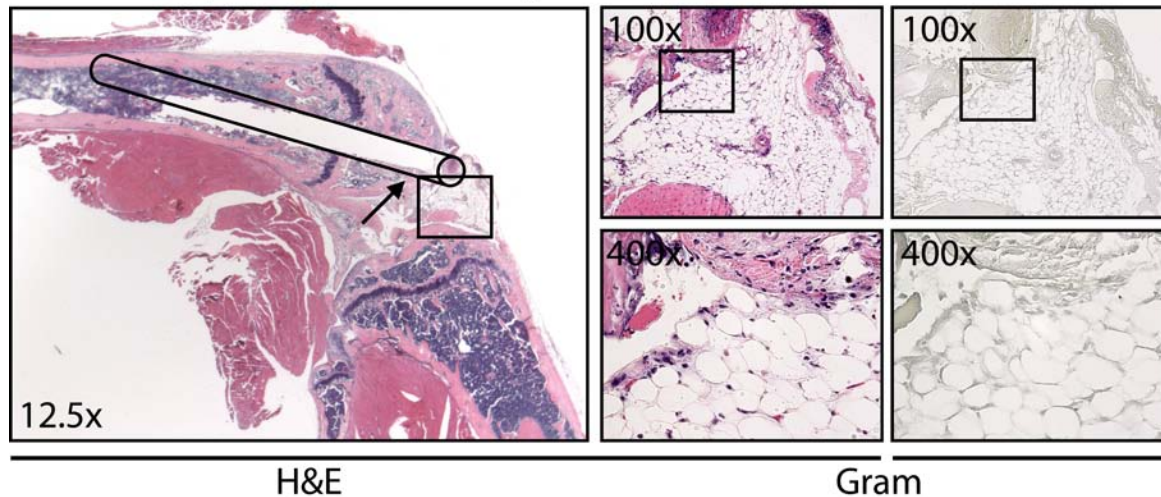


Neutrophils admixed with gram-positive bacteria can be detected in the joint tissue surrounding the implant after *S. aureus* infection

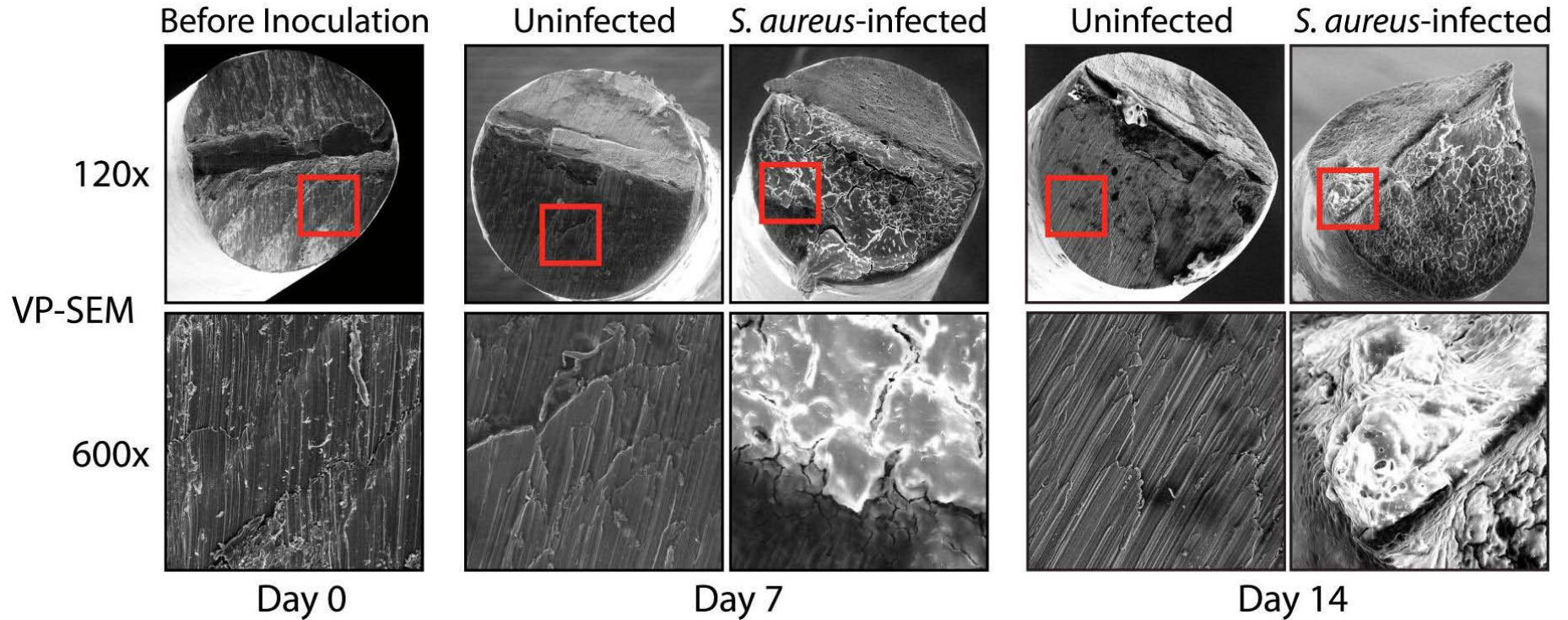
S. aureus-infected post-operative knee joint



Uninfected post-operative knee joint



Biofilm formation was readily observed on the metallic implants after post-operative *S. aureus* infection



Proof of Concept -- Infection is exclusively on intra-articular metal

QuickTime™ and a
YUV420 codec decompressor
are needed to see this picture.

2009

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September 2010 | Volume 5 | Issue 9 | e12580

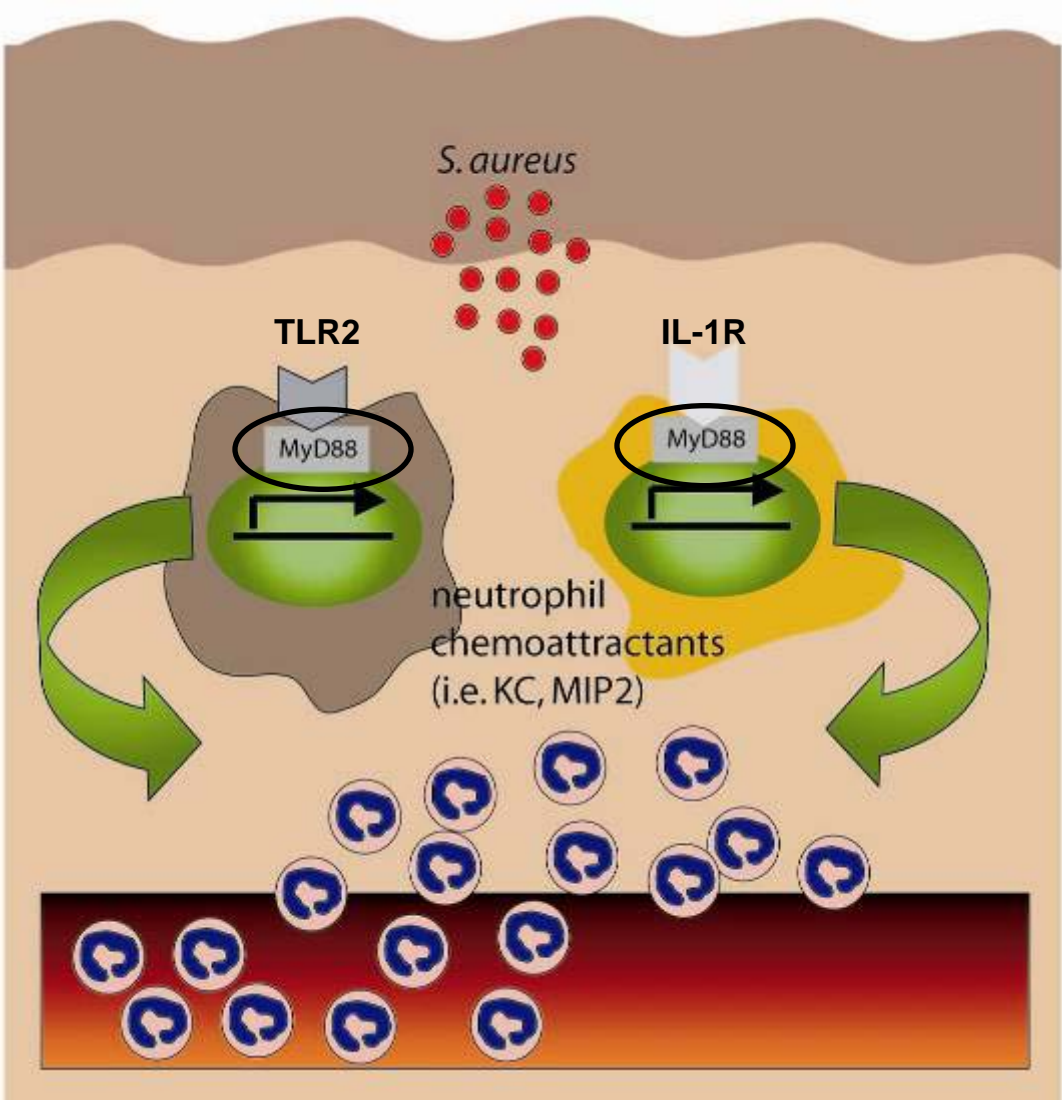
2010

To use this model to better define the protective innate immune mechanisms

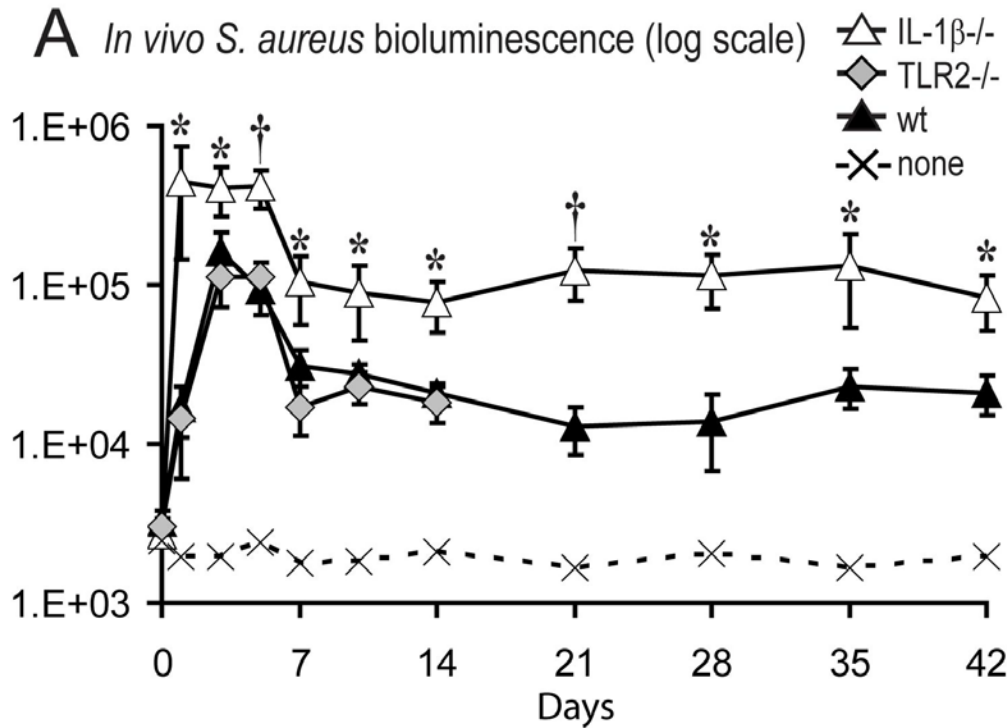
To use this model to investigate the effectiveness of antibiotic and antimicrobial implant coatings

To evaluate current metals for differences in susceptibility to infection

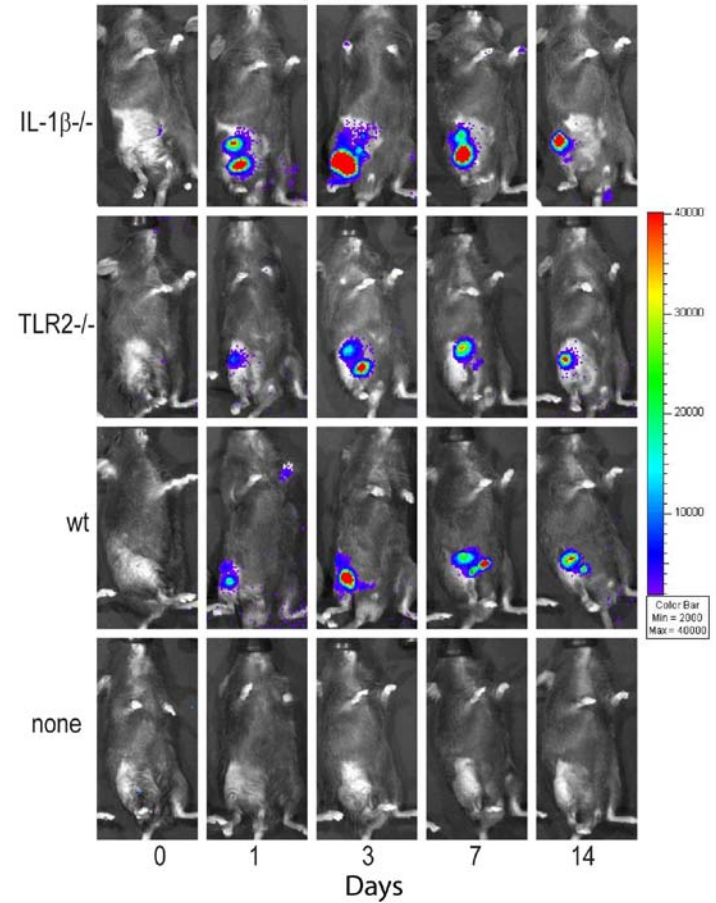
Proposed role of MyD88 in mediating neutrophil recruitment



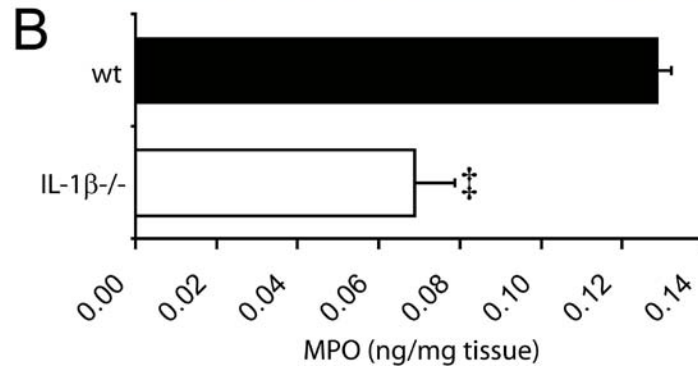
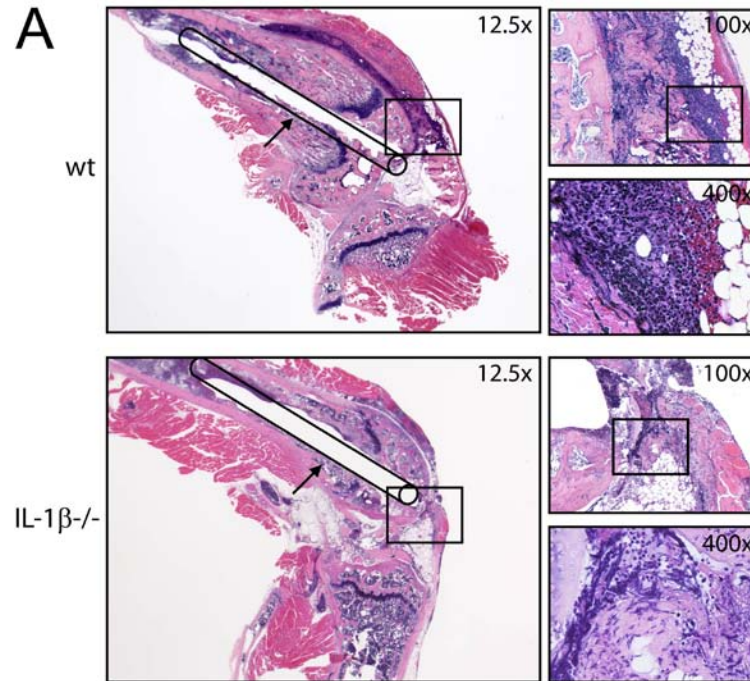
IL-1 β -deficient mice had increased *in vivo* bacterial burden compared with TLR2-deficient mice or wt mice.



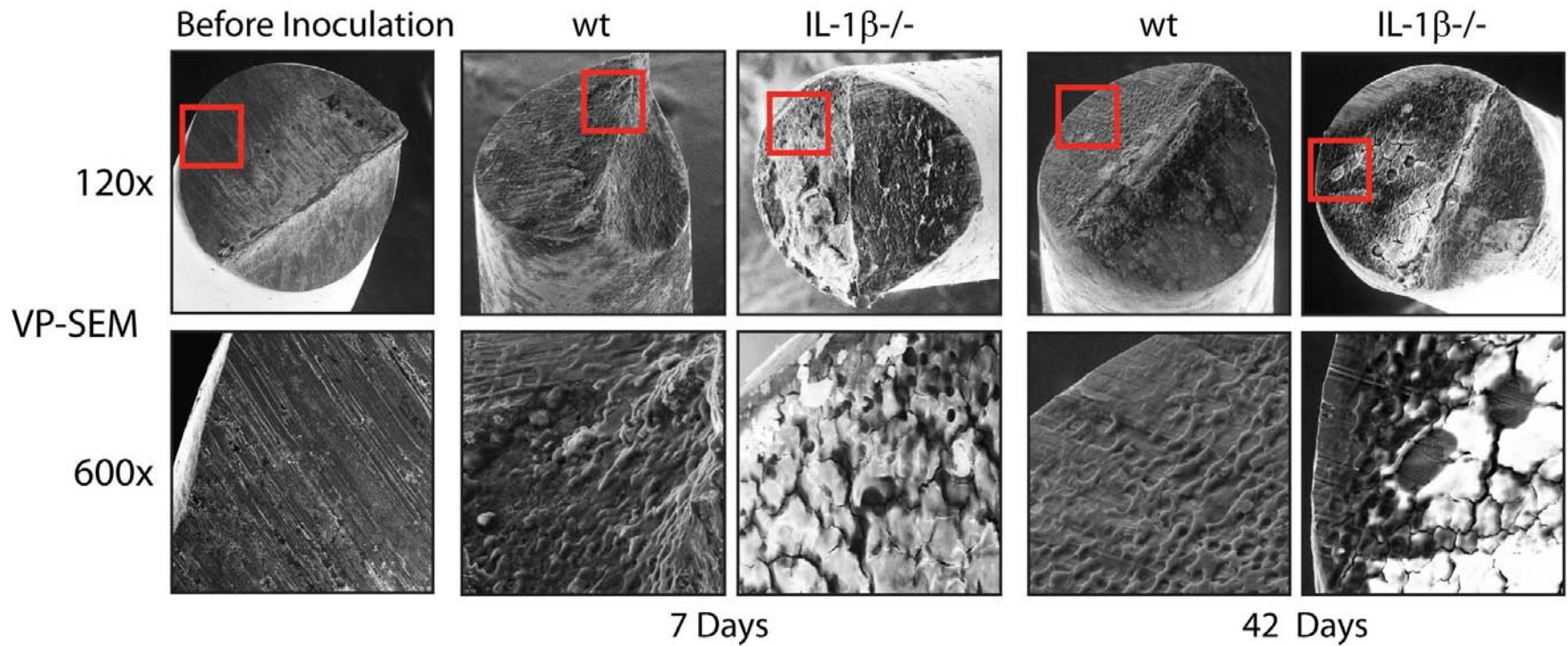
B *In vivo S. aureus* bioluminescence representative images (color scale)



IL-1 β -deficient mice had decreased neutrophil recruitment to the infected knee-joints compared with wt mice.



IL-1 β -deficient mice had substantially more biofilm formation on the implants than wt mice.



Conclusions

IL-1 β -deficient mice had:


- **Increased *in vivo* bacterial burden**
- **Increased biofilm formation**
- **Decreased neutrophil recruitment**

Taken together, these findings suggest that IL-1 β may play an integral role in the host immune response to infection after total joint replacement by promoting neutrophil recruitment.

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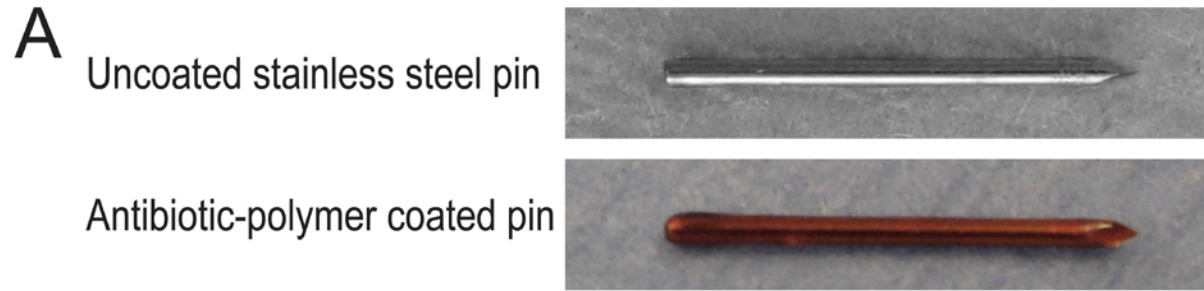
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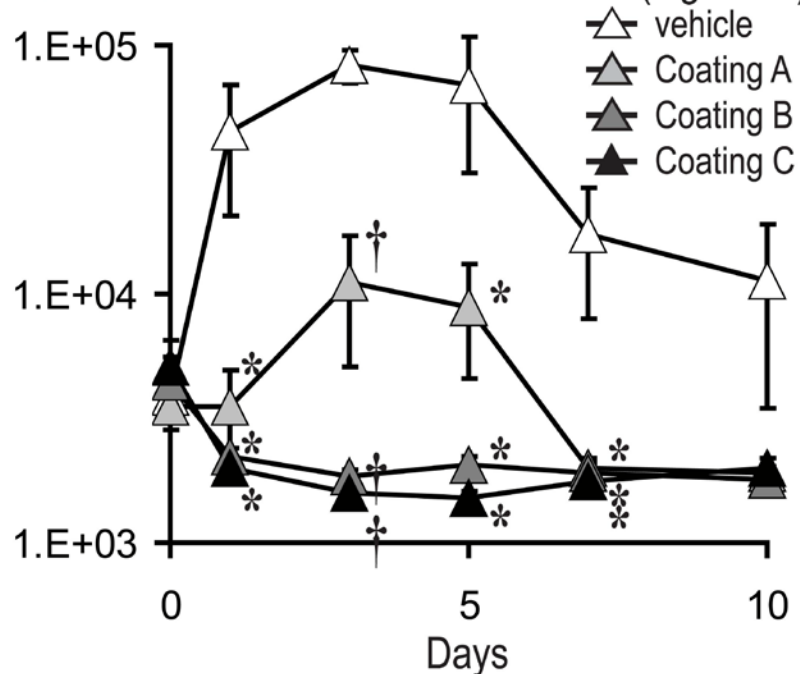
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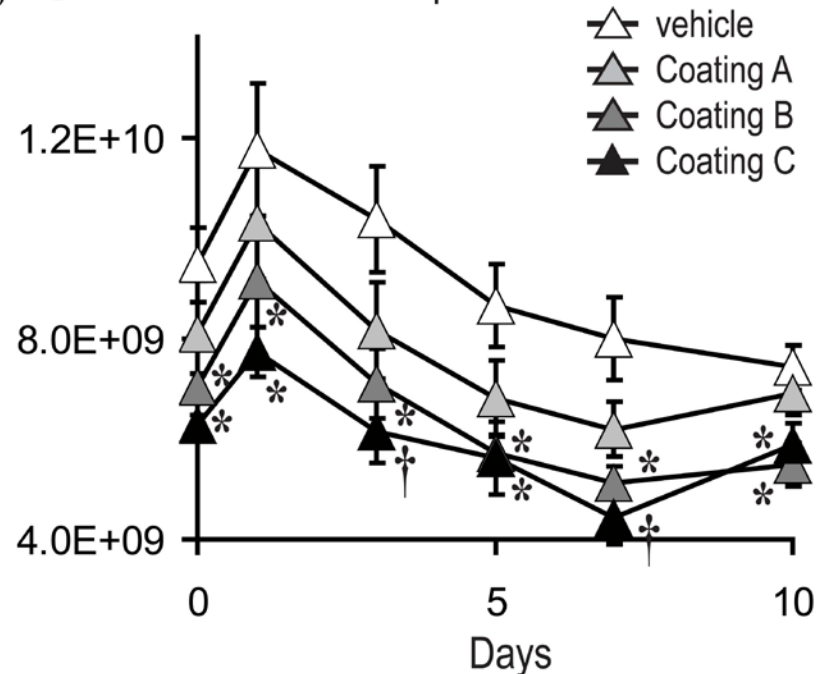
A novel antibiotic-impregnated implant coating results in reduced *S. aureus* infection and decreased inflammation



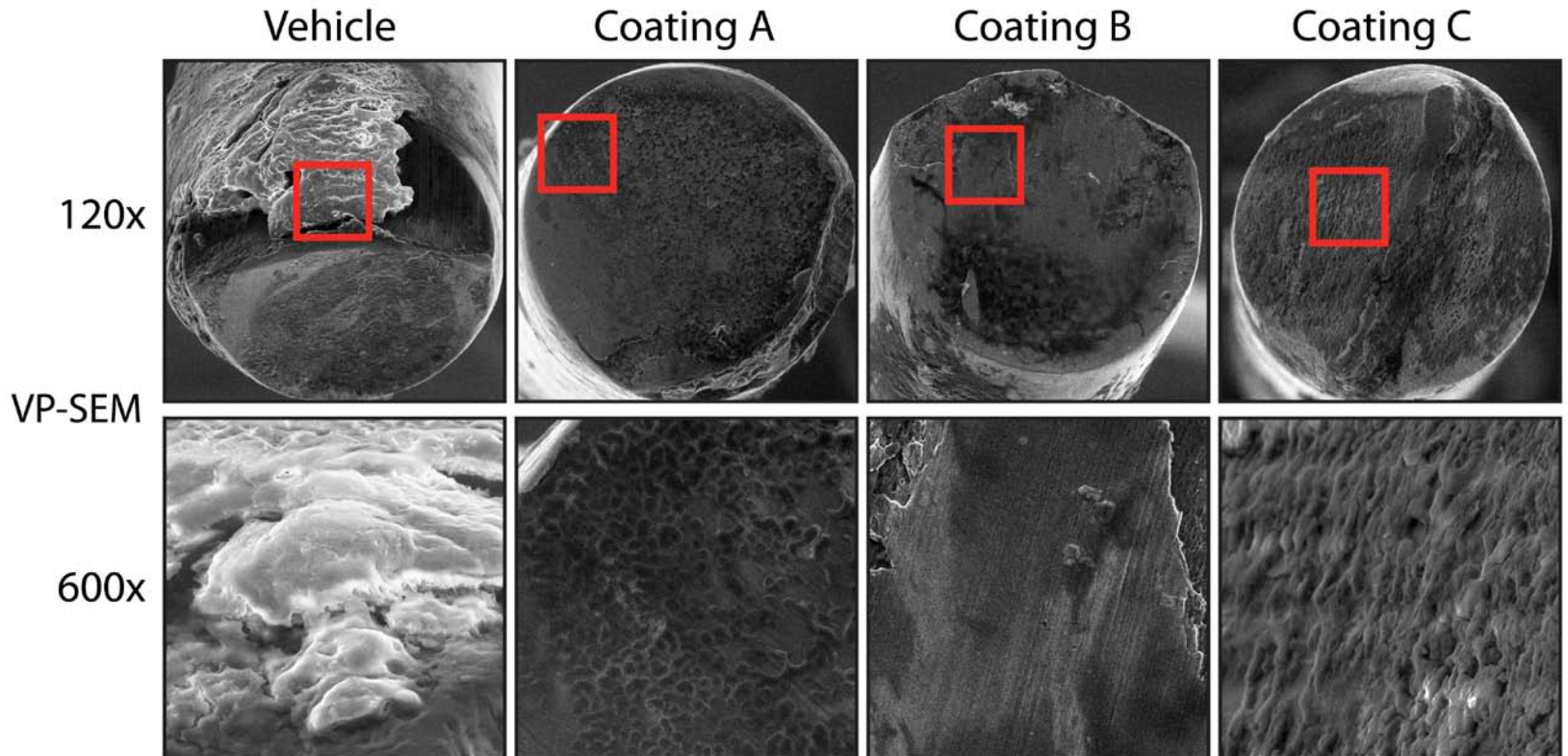
B *In vivo* *S. aureus* bioluminescence (log scale)



C *In vivo* EGFP neutrophil fluorescence



A novel antibiotic-impregnated implant coating results in reduced *S. aureus* biofilm formation



Conclusions

Antibiotic coated pins had:

- **Decreased *in vivo* bacterial burden to baseline**
- **Decreased biofilm formation**

Taken together, these findings suggest that antibiotic/antimicrobial coatings hold significant promise in the prevention of post-arthroplasty joint infection

Future Directions and Final Thoughts

- **Implant infections are disastrous complications for ALL surgeons**
- ***In Vivo* bioluminescence offers a non-invasive, efficient means to LONGITUDINALLY track infections**
- **Bacterial panels will be key**
- **Understanding host biology is essential**
- **Implant coatings are the holy grail!**

Special Thanks



UCLA Orthopaedic Hospital Research Center

John Adams, M.D.

Daniel Uslan, M.D.



UCLA Crump Institute of Molecular Imaging

David Stout, PhD



Dartmouth Medical School

Ambrose Cheung, PhD



Department of Biomedical Engineering, UC. Davis

Scott Simon, Ph.D.



TyRx Pharma, Inc.

Arikha Moses, Ph.D.

Satish Pulapura, Ph.D.

Qing Ge, Ph.D.