

Modulating Micrococcal Nuclease-Triggered Antibiotic Releases for Improved Outcomes of One-Stage Revisions of *Staphylococcus aureus* Infected Prostheses

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INTRODUCTION: While the rate of primary orthopedic implant associated infection is relatively low, the reinfection rate post-implant revision of previously infected prostheses can be as high as 30%. *Staphylococcus aureus* (*S. aureus*) is the predominant pathogen responsible for orthopedic implant-related infections and revision failures. Existing prophylactic and therapeutic strategies fail to consistently eradicate infections following revisions even with aggressive local and systemic antibiotic treatments. We previously developed hydrogel coatings for intramedullary (IM) metallic pins where antibiotics were covalently tethered to a polyethylene glycol dimethacrylate (PEGDMA)-based hydrogel via an oligonucleotide (oligo) linker (5'-carboxy-mCmGTTmCmG-3'-acryd, 0PS; Figure 1A).^{1,2} When these pins were implanted in *S. aureus*-inoculated rat femoral canals, on-demand release of antibiotics from the coating, due to rapid cleavage of the oligo linker by micrococcal nuclease (MN) secreted by the bacteria, prevented infections. Here we test the hypothesis that tethering ampicillin to the implant coating by combining unmodified oligo linker (0PS, rapid MN cleavage) with phosphorothioate-modified linker (6PS, more sustained MN cleavage) could effectively mitigate or eradicate infections following one-stage revisions of previously infected prostheses, with/without a single dose of systemic vancomycin at the time of revision.

METHODS: Ti6Al4V pins ($\phi=0.5$ mm; 25-mm long) were first coated with N-(3,4-dihydroxyphenethyl) methacrylamide (DopaMA) and then UV-crosslinked with PEGDMA hydrogel coatings with/without 150 μ M of 0PS, 6PS, or a 1:1 mixture of 0PS and 6PS. Next ampicillin was covalently attached to the tethered oligo linkers via amidation upon EDC/sNHS activation resulting in coated pins (0PS-Amp, 6PS-Amp, 0&6PS-Amp, or Ctrl). The on-demand release of ampicillin from these coated pins was validated with a clear zone assay on bioluminescent Xen29 *S. aureus* agar plates (Figure 1B). All animal experiments were approved by institutional IACUC. Male SASCO SD rats were used for consistent skeletal size. As shown in Scheme 1, during the primary surgery, 200 CFU Xen29 *S. aureus* or LB broth was injected into the rat femoral canals followed by the insertion of an uncoated IM pin. A one-stage revision was performed on day 3 including removal of the primary pin, debridement of the IM canal, and insertion of a revision IM pin coated with Ctrl, 0PS-Amp, 6PS-Amp, or 0&6PS-Amp hydrogel. A subset of rats received an intraperitoneal (IP) vancomycin injection (55 mg/kg) immediately post revision. Rats were monitored with longitudinal microcomputed tomography (μ CT) and sacrificed 3 weeks post revision. Explanted femurs with pins in place were imaged with IVIS, and after pin retrieval, bacterial loads on sonicated crushed femurs and pins were quantified.

RESULTS: μ CT confirmed femoral infections characterized by cortical thickening and bone lesions following one-stage revision with Ctrl-hydrogel coated pins, but detected no morphological (Figure 2A) or quantitative changes in bone volume fraction (BVF) or cortical thickness (C. Th) (Figure 2B) in those treated with 0PS-Amp, 6PS-Amp or 0&6PS-Amp-coated pins compared to the uninfected control. IVIS imaging of explanted femurs at 3 weeks post-revision, however, still detected signals from bioluminescent *S. aureus* in the groups receiving 0PS-Amp and 6PS-Amp but not the 0&6PS-Amp coatings (Figure 2C). Quantification of bacteria in the crushed femurs and on retrieved pins confirmed that the 0&6PS-Amp treatment led to more consistent mitigation of bacterial burdens compared to 0PS-Amp and 6PS-Amp treatments (Figure 2D). When a single IP injection of vancomycin post one-stage revision was combined with the 0&6PS-Amp coating, bacteria were eradicated, outperforming the coating alone or vancomycin injection alone as shown by μ CT (Figure 2E) and bacterial load quantifications (Figure 2F).

DISCUSSION: The 0&6PS-Amp coating applied to the revision pin enabled timely elimination of residual planktonic bacteria both in the debrided tissue compartment and migrating out of surrounding tissues via the more sustained MN-triggered release of ampicillin following a one-stage revision of Xen29 infected IM pins. When combined with a single systemic vancomycin, this coating nearly eradicated bacteria following the one-stage revision.

SIGNIFICANCE/CLINICAL RELEVANCE: The near eradication of infection achieved with the 0&6PS-Amp coating in combination with a single systemic vancomycin injection at the time of revision is clinically and translationally significant as extended systemic antibiotic therapy is a common practice following revisions of previously infected prostheses. It is perceivable that eradication could be achieved with additional systemic vancomycin.

REFERENCES: [1] Skelly JD, et al. (2023). *ACS Appl Mater Interfaces*, 15, 37174. [2] Chen, F, et al. (2024). *ACS appl mater interfaces*, 16, 24421.

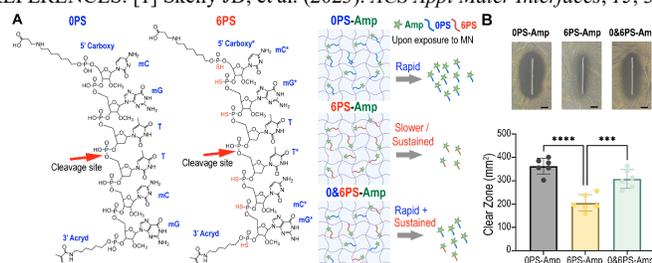
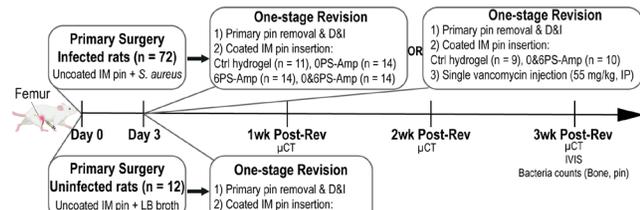


Figure 1. (A) Structures of oligo linkers 0PS, 6PS, and coatings with rapid (0PS-Amp), sustained (6PS-Amp), rapid+sustained (0&6PS-Amp) MN-triggered ampicillin releases. (B) Images and quantification (n=5) of clear zones around coated IM pins on *S. aureus* agar plates, Bars: 5 mm.



Scheme 1. Study design for determining efficacy of MN-triggered ampicillin release coatings \pm a single vancomycin injection.

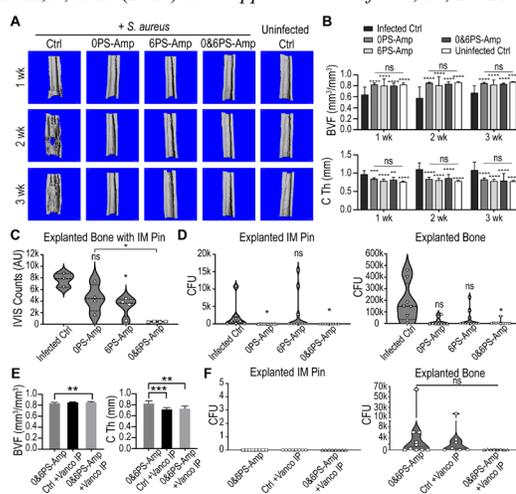


Figure 2. (A) 3D μ CT images & (B) longitudinal quantifications (n=8-14) of femoral BVF & C. Th. (C) IVIS bioluminescence of explanted femurs with IM pins 3 weeks post revision (n=3-4). (D) Bacterial load on explanted IM pins and in crushed femurs (n=8-11) 3 weeks post revision. (E) μ CT (n=9-10) & (F) bacterial loads on explanted IM pins (n=8-9) & in crushed femurs (n=7-10) 3 weeks post revision with 0&6PS-Amp \pm vancomycin.