Phage-Augmented Implant Retention for Polymicrobial Spinal and Fracture-Fixation Hardware Infection: Exploratory De-Risking of Personalized Lytic Cocktails as a Biofilm-Targeting Adjunct to DAIR

NIAMS · R21 (Exploratory/Developmental). Concept for discussion — illustrative figures are flagged [ILLUSTRATIVE]; not a submitted application.

Project Summary / Abstract

Infections of spinal instrumentation and fracture-fixation hardware are among the most refractory implant-associated infections in orthopedic and spine surgery. Bacteria colonize titanium and steel as antibiotic-tolerant biofilms, and when the implant cannot be removed without sacrificing spinal stability or fracture union, surgeons depend on prolonged, frequently failing suppressive antibiotics. These infections are often polymicrobial — combining *Staphylococcus aureus*/*epidermidis*, *Cutibacterium acnes*, *Pseudomonas aeruginosa*, and Enterobacterales — which compounds resistance and biofilm tolerance. Lytic bacteriophages are mechanistically matched to this niche: they self-amplify at the infection site, penetrate and degrade biofilm matrix, kill metabolically dormant biofilm-embedded cells that antibiotics miss, and can be assembled into multi-phage cocktails covering several co-pathogens while sparing commensals. Early human experience supports feasibility but not efficacy: a 2025 case report documented sustained remission off antibiotics after 35 IV phage doses for implant-associated MRSA spondylodiscitis with non-removable instrumentation (Arientová et al., 2025), while a controlled 2025 sheep fracture-related infection (FRI) model found phage plus vancomycin was safe but did **not** significantly reduce bacterial burden, limited by rapid phage clearance and up to 99.9% neutralization (Peez et al., 2025). **Central hypothesis:** a phagogram-guided, polymicrobial lytic cocktail, delivered to overcome the clearance/neutralization barrier, can measurably reduce biofilm burden on retained hardware as an adjunct to debridement, antibiotics, and implant retention (DAIR) — but only if route, dosing, and synergy are optimized first. This R21 performs focused exploratory de-risking: we will (1) assemble and characterize cocktails active against patient-derived polymicrobial biofilms on titanium; (2) define phage–antibiotic synergy and

the pharmacokinetic/neutralization barriers in a controlled FRI model, comparing IV versus local delivery; and (3) build the phagogram, regulatory, and clinical-readiness framework for a future investigator-initiated trial under an emergency/expanded-access IND. The work is high-risk/high-reward, aligns with NIAMS musculoskeletal-infection priorities, and is responsive to DoD CDMRP trauma interests.

Specific Aims

Spinal and fracture-fixation hardware infections frequently cannot be cured without removing the implant, yet removal sacrifices the stability the hardware provides until bone heals. Polymicrobial biofilms on metal tolerate antibiotics, and current salvage relies on debridement, antibiotics, and implant retention (DAIR) with chronic suppression that often fails. Lytic phage cocktails offer a biofilm-active, species-tunable adjunct, but existing evidence is confined to preclinical models and compassionate-use case reports — and the one controlled large-animal FRI study found that phage added to vancomycin did not, by itself, lower bacterial burden (Peez et al., 2025). This exploratory project does not assume efficacy; it tests whether the barriers that blocked efficacy can be overcome, and whether a personalized polymicrobial cocktail approach can be rationally assembled, optimized, and made trial-ready.

Central hypothesis. A phagogram-guided polymicrobial lytic cocktail, paired with standard-of-care antibiotics and delivered to counter rapid clearance/neutralization, will reduce viable biofilm on retained titanium hardware more than antibiotics alone — establishing a credible, trial-ready, implant-sparing adjunct to DAIR.

Aim 1 — Assemble and characterize personalized lytic phage cocktails against polymicrobial hardware-biofilm isolates on titanium. Using banked clinical polymicrobial isolates (e.g., *S. aureus*/*epidermidis* with *P. aeruginosa* or *C. acnes*), we will construct phagograms, build 2–4-phage cocktails, and quantify biofilm disruption on titanium discs that model the implant surface (extending the titanium-disc *C. acnes* model of Chen et al., 2024 to polymicrobial communities). We will test whether cocktails outperform single phages and suppress phage-resistant mutant emergence.

Aim 2 — Define phage–antibiotic synergy, pharmacokinetics, and neutralization in a controlled FRI model. In an established plate-fixation FRI model, we will compare IV versus local phage delivery, each combined with standard-of-care antibiotic, against antibiotic alone, with implant retention throughout — measuring bacterial burden on hardware/bone, biofilm clearance, phage clearance kinetics, and neutralizing-antibody responses. This aim directly interrogates the clearance/neutralization barrier that limited efficacy in prior controlled work (Peez et al., 2025).

Aim 3 — Establish the phagogram pipeline and regulatory/clinical-readiness framework for a

future investigator-initiated trial. We will time the swab-to-phagogram-to-cocktail workflow against the Aim 1 library; draft the FDA emergency/expanded-access IND (eIND) pathway and IRB framework; and pre-specify endpoints, safety/neutralization monitoring, and feasibility/stopping criteria for a last-resort, implant-sparing protocol.

Impact. If the clearance/neutralization barrier can be overcome and adjunctive benefit demonstrated, this project converts anecdotal salvage into a rationally optimized, trial-ready, implant-sparing phage protocol that could break the biofilm stalemate at the metal–bone interface without the morbidity of staged hardware removal. If the barrier proves decisive, the project still delivers a definitive, well-controlled answer plus a reusable phagogram/regulatory framework — a high-value outcome for an exploratory award.

Significance

Hardware-associated infection is a major driver of morbidity, reoperation, and cost in spine and trauma surgery, and the central clinical dilemma is mechanical: spinal instrumentation maintains alignment and fracture-fixation hardware maintains union, so removal to eradicate infection is often unacceptable until bone has healed. When the implant must stay, management defaults to DAIR with chronic suppression, which is frequently inadequate because biofilms on titanium and steel tolerate antibiotics and shelter metabolically dormant cells. The problem is compounded by polymicrobial ecology — co-infection by *S. aureus*/*epidermidis*, *C. acnes*, *P. aeruginosa*, and Enterobacterales — that broadens resistance and deepens biofilm tolerance. FRI in particular remains a management challenge for which adjunctive strategies, including bacteriophage therapy for drug-resistant organisms, have been proposed but not standardized (Foster et al., 2020). [ILLUSTRATIVE] A meaningful fraction of implant-retention salvage attempts fail and proceed to reoperation or lifelong suppression; precise local incidence, failure, and cost figures will be cited from institutional and registry data in the full application.

Lytic phages address the specific mechanisms that make hardware infection recalcitrant. They self-amplify at the infection site, penetrate and degrade biofilm extracellular matrix via phage-encoded depolymerases and lysins, kill dormant biofilm-embedded cells that antibiotics miss, and — assembled as cocktails — cover multiple co-pathogens while sparing commensals. Phage–antibiotic synergy can re-sensitize biofilm bacteria to drugs they otherwise tolerate, and cocktails plus synergy can suppress phage-resistant mutants. Proof-of-concept for the implant niche exists: an optimized cocktail disrupted *C. acnes* biofilm and reduced biofilm biomass on titanium discs mimicking the device environment (Chen et al., 2024), and a regulator-approved course of 35 IV phage doses (10^9 PFU) achieved sustained remission off antibiotics at six months in implant-associated MRSA spondylodiscitis with non-removable instrumentation (Arientová et al., 2025).

Critically, the evidence also defines the barrier this project must clear. The only controlled large-animal FRI study to date found that phage added to vancomycin was safe but did **not** significantly change bacterial load, with phage undetectable in circulation by 240 minutes and neutralization reaching ~99.9% (Peez et al., 2025). This is the pivotal motivation for an *exploratory* award: the open question is not whether phages can lyse these organisms in a dish — they can — but whether delivery, dosing, and synergy can be engineered so that biofilm-active benefit survives in vivo on retained hardware. NIAMS-relevant impact is therefore high and well-scoped: a validated, implant-sparing adjunct would change salvage of musculoskeletal hardware infection, and a rigorous negative result would prevent futile clinical adoption.

Innovation

This project is innovative in four respects. First, it targets *polymicrobial* hardware biofilms explicitly, designing cocktails to cover co-infecting species simultaneously rather than treating a single pathogen — the realistic clinical scenario for spinal and fracture hardware. Second, it tests a *combined local-plus-systemic, implant-sparing* delivery concept on the actual metal–bone interface using titanium-disc biofilm models and a plate-fixation animal model, rather than planktonic assays. Third, and most distinctively, it elevates the principal failure mode identified in prior controlled work — rapid phage clearance and host neutralization — from a footnote to the *primary experimental variable*, comparing routes, repeated/local dosing, and synergy rather than presupposing efficacy. Fourth, it treats *trial-readiness itself as a deliverable*, building the operational and regulatory scaffolding (rapid phagogram turnaround, eIND/expanded-access pathway, IRB framework, pre-specified endpoints) that any future personalized-phage trial will require. The approach deliberately stays within current evidence: it positions phage cocktails as a biofilm-cracking adjunct to DAIR and antibiotics, while noting that engineered/CRISPR-armed phages and depolymerase enzybiotics remain largely preclinical for hardware infection and are out of scope here.

Approach

Aim 1 — Assemble and characterize personalized lytic phage cocktails against polymicrobial hardware-biofilm isolates on titanium

Rationale. Cocktails covering multiple co-pathogens, guided by phagograms, are the basis of personalized therapy, and biofilm disruption on titanium is the relevant readout for hardware infection. Chen et al. (2024) disrupted *C. acnes* biofilm and reduced biomass on titanium discs; we extend that validated platform to polymicrobial communities.

Experimental design. From banked, de-identified clinical isolates representing polymicrobial

hardware infections (e.g., *S. aureus*/*epidermidis* + *P. aeruginosa*; *S. aureus* + *C. acnes*), we will generate phagograms against a curated lytic phage library, then build 2–4-phage cocktails. Single-species and defined mixed-species biofilms will be grown on standardized titanium discs and exposed to single phages, cocktails, and cocktail-plus-antibiotic conditions. Outcomes: viable biofilm CFU, matrix/biomass quantification (e.g., confocal imaging as in Chen et al., 2024), and longitudinal monitoring for phage-resistant-mutant outgrowth and any antibiotic re-sensitization (synergy). [ILLUSTRATIVE] target: ≥10 polymicrobial isolate pairs screened; ≥3 candidate cocktails advanced to Aim 2.

Expected outcomes. Cocktails reduce polymicrobial biofilm burden on titanium more than single phages and delay/suppress resistant-mutant emergence, yielding 1–3 lead cocktails with defined host range for animal testing.

Potential pitfalls & alternatives. Some clinical strains may lack matched phages; we will prioritize broad-host-range phages, expand the library, and report coverage gaps as a planning output for Aim 3. If interspecies competition confounds mixed biofilms, we will use defined dual-species models with sequential, species-resolved readouts. Because all in vitro outcomes are quantitative and self-contained, Aim 1 generates a complete, publishable dataset even if Aim 2 is delayed.

Aim 2 — Define phage–antibiotic synergy, pharmacokinetics, and neutralization in a controlled FRI model

Rationale. A controlled plate-fixation FRI model is the most clinically relevant preclinical system and is precisely where the clearance/neutralization barrier was quantified and where phage failed to lower bacterial load as an adjunct (Peez et al., 2025). Resolving route-of-delivery and synergy questions here is prerequisite to any trial, and directly tests whether that barrier is surmountable.

Experimental design. Using an established tibial-osteotomy/plate-fixation FRI model with a drug-resistant *S. aureus* challenge, animals receive standard-of-care antibiotic (e.g., vancomycin) with: (a) IV phage, (b) local phage, or (c) antibiotic alone, with implant retention throughout. Endpoints: bacterial burden on hardware and bone; biofilm clearance with the implant retained; serial phage titers (clearance kinetics); and neutralizing-antibody development. Dosing and schedules are explicitly designed to *counter* the prior failure mode — e.g., repeated and/or local dosing and cocktail composition chosen to offset rapid clearance — not to replicate a single-bolus IV regimen. Group sizes and schedules set with veterinary/biostatistics input. [ILLUSTRATIVE] 3 arms; per-group n by power analysis; phage dosing in the ~10^9 PFU range informed by prior human/animal reports (Arientová et al., 2025; Peez et al., 2025).

Expected outcomes. We expect phage + antibiotic to be safe (consistent with Peez et al., 2025) and, with clearance-aware dosing, to show route-dependent differences in phage persistence and

neutralization. The *primary* prespecified question is whether any route/dosing strategy yields a statistically meaningful reduction in biofilm/bacterial burden on retained hardware versus antibiotic alone. We treat a null result as an informative, decision-grade outcome: prior controlled data (Peez et al., 2025) showed no burden reduction, so confirming or overturning that under optimized delivery is itself the deliverable.

Potential pitfalls & alternatives. Rapid clearance and neutralization may again blunt IV efficacy; mitigations include local/repeated dosing, cocktail rotation, and synergy optimization carried over from Aim 1. If the large-animal model is rate-limiting or cost-limiting within the R21 envelope, the same prespecified questions will be addressed first in a small-animal osteomyelitis model, with large-animal work scoped to the single most informative comparison. Phages remain an adjunct; no arm tests phage monotherapy as a cure.

Aim 3 — Establish the phagogram pipeline and regulatory/clinical-readiness framework for a future investigator-initiated trial

Rationale. Personalized phage therapy depends on rapid swab-to-cocktail matching and a defined regulatory route. Last-resort musculoskeletal phage therapy is already being delivered and registered prospectively (e.g., the PHAGEFORCE registry, ClinicalTrials.gov NCT06368388, cited here only as a real-world feasibility pointer, not as a scientific reference), demonstrating that the operational model is achievable.

Experimental design. We will (1) prototype and time the phagogram-to-cocktail workflow against the Aim 1 library to set turnaround targets; (2) draft the FDA emergency/expanded-access IND (eIND) pathway and single-patient/expanded-access procedures for investigational phage, including magistral-style production considerations; and (3) pre-specify a last-resort implant-sparing protocol — eligibility, combined local + IV dosing alongside DAIR, safety monitoring (including neutralization surveillance), endpoints, and feasibility/stopping criteria — with IRB framework and registry-style data capture. [ILLUSTRATIVE] phagogram turnaround target: days, not weeks; planning-cohort framing only, no enrollment in this R21.

Expected outcomes. A documented, IRB-ready protocol and regulatory package, plus realistic turnaround and coverage metrics, positioning a subsequent investigator-initiated trial.

Potential pitfalls & alternatives. Regulatory timelines and product-quality requirements are demanding; we will engage FDA early via a pre-submission meeting and seek partnership with an established magistral/clinical phage program. If domestic production access is limiting, we will define candidate partnerships and document the gap as a key planning finding.

Timeline

[ILLUSTRATIVE] Two-year R21. **Months 1–12:** Aim 1 phagogram/cocktail assembly and titanium-disc biofilm assays; initiate Aim 3 regulatory drafting and phagogram-workflow prototyping (Aims 1 and 3 run in parallel and are independent of animal work). **Months 9–24:** Aim 2 studies (staggered cohorts) once ≥1 lead cocktail is selected; small-animal confirmation precedes any large-animal scale-up. **Months 18–24:** Integrate Aim 2 PK/neutralization data into the Aim 3 protocol and regulatory package; finalize trial-readiness deliverables. **Go/no-go (~Month 12):** ≥1 lead cocktail with quantified titanium-biofilm activity advances to animal testing; if none meets threshold, resources shift to deepening Aim 1 coverage and the Aim 3 framework.

Budget Justification

[ILLUSTRATIVE] Modular R21 within NIAMS limits (NIH R21: up to **$275,000 direct costs total over two years**; figures here are placeholders). Because a full large-animal program can exceed an R21 envelope, Aim 2 is deliberately scoped — small-animal confirmation first, then a single most-informative large-animal comparison — so the science fits the mechanism.

- **Personnel [ILLUSTRATIVE].** PI(s) effort for scientific direction; microbiology technician/postdoc for phagogram and biofilm assays (Aim 1); animal-study coordination (Aim 2); regulatory/clinical-research coordinator (Aim 3).
- **Animal costs [ILLUSTRATIVE].** FRI-model procurement, surgery, husbandry, imaging, and veterinary oversight (Aim 2) — the largest line; scoped as above to remain feasible.
- **Supplies [ILLUSTRATIVE].** Titanium discs, phage propagation/purification, antibiotics, biofilm/microbiology consumables, sequencing for host-range/resistance.
- **Other [ILLUSTRATIVE].** Biostatistics, FDA pre-submission/regulatory consulting, phage-production partnership costs. Exact figures to be finalized with the institutional grants office; all amounts above are [ILLUSTRATIVE].

Vertebrate Animals

Animal work is proposed only in Aim 2. A controlled FRI model (tibial osteotomy with plate fixation), consistent with the established sheep FRI paradigm (Peez et al., 2025), will evaluate IV versus local phage plus antibiotic with implant retention. Justification: biofilm clearance on retained hardware and phage clearance/neutralization kinetics cannot be assessed in vitro. Procedures will follow IACUC-approved protocols with veterinary oversight, appropriate anesthesia/analgesia, and defined humane endpoints. **Reduction/refinement:** small-animal osteomyelitis models will address confirmatory questions before any large-animal scale-up, and group sizes [ILLUSTRATIVE] will be

set by power analysis to use the minimum number of animals required for statistical validity.

Human Subjects / Clinical Trial

No human-subjects enrollment or clinical trial is conducted in this R21. Clinical work is limited to *planning* a future investigator-initiated study (Aim 3) and to use of de-identified banked clinical isolates (Aim 1), which will undergo IRB review for human-subjects determination. Aim 3 will pre-specify the framework for a subsequent last-resort, implant-sparing protocol — including the FDA emergency/expanded-access IND (eIND) route for investigational phage (single-patient eIND and expanded-access procedures), IRB oversight, informed consent, safety monitoring (including phage-neutralization surveillance), and registry-style data capture aligned with prospective real-world practice (e.g., NCT06368388). Any future treatment use of phage in patients will proceed only under appropriate FDA authorization and IRB approval.

Team & Environment

This project requires an interdisciplinary team; roles below are templates to be filled with named investigators and institutions.

- **Contact PI [name, institution] — phage biology/microbiology:** phagogram pipeline, cocktail assembly, titanium-biofilm assays (Aim 1).
- **MPI/Co-I [name, institution] — orthopedic/spine surgeon-scientist:** clinical relevance, implant-retention/DAIR design, isolate sourcing, trial framework (Aims 2–3).
- **Co-I [name, institution] — preclinical orthopedic-infection/animal model:** FRI studies and PK/neutralization (Aim 2).
- **Co-I [name, institution] — infectious disease/regulatory:** eIND/expanded-access pathway, safety monitoring (Aim 3).
- **Consultants:** biostatistics; FDA regulatory affairs; clinical phage-production partner.
- **Environment:** institutional microbiology and biofilm laboratories; AAALAC-accredited animal facility; IRB/IACUC; access to clinical-isolate biobanks and a curated phage library. Engagement with an established phage-therapy program for benchmarking and potential production partnership is anticipated (not yet secured) and will be documented as part of the Aim 3 readiness deliverable.

References

1. Arientová S, Beran O, Belšan T, Holub M. Successful systemic phage therapy for implant-

associated MRSA spondylodiscitis. *International Journal of Infectious Diseases.* 2025;164:108357. https://pubmed.ncbi.nlm.nih.gov/41478341/

2. Peez C, Chen B, Henssler L, et al. Evaluating the safety, pharmacokinetics and efficacy of phage therapy in treating fracture-related infections with multidrug-resistant *Staphylococcus aureus*: intravenous versus local application in sheep. *Frontiers in Cellular and Infection Microbiology.* 2025;15:1547250. https://pubmed.ncbi.nlm.nih.gov/40256450/
3. Chen B, Chittò M, Tao S, Wagemans J, Lavigne R, Richards RG, Metsemakers WJ, Moriarty TF. Isolation and Antibiofilm Activity of Bacteriophages against *Cutibacterium acnes* from Patients with Periprosthetic Joint Infection. *Viruses.* 2024;16(10):1592. https://pubmed.ncbi.nlm.nih.gov/39459925/
4. Foster AL, Moriarty TF, Trampuz A, et al. Fracture-related infection: current methods for prevention and treatment. *Expert Review of Anti-infective Therapy.* 2020;18(4):307-321. https://pubmed.ncbi.nlm.nih.gov/32049563/

Trial-registry pointer (not a scientific reference): PHAGEFORCE registry, ClinicalTrials.gov NCT06368388.