Implant-Sparing Bacteriophage Therapy for *Staphylococcus aureus* Surgical-Site and Mesh Infections: Cocktail Design, Phage–Antibiotic Synergy, and a Rapid Susceptibility Pipeline

Funding mechanism: NIH/NIAID R21 (Exploratory/Developmental). **Project period:** 2 years. **Costs:** modular, within the R21 cap of $275,000 direct over two years (≤$200,000 in any single year).

Project Summary / Abstract

Surgical-site infections (SSIs) involving implanted mesh, vascular grafts, and other prosthetic material are a major driver of surgical morbidity, and *Staphylococcus aureus* — including methicillin-resistant strains (MRSA) — is the dominant pathogen. Once *S. aureus* colonizes a foreign body it forms a recalcitrant biofilm on the device surface that shields it from antibiotics and host immunity, so cure frequently requires surgical explantation, with attendant reoperation risk, cost, and morbidity. Lytic bacteriophages are mechanistically suited to this problem: they self-amplify at the site of infection, penetrate and disrupt biofilm matrix, reach metabolically dormant persister cells, and remain active against multidrug-resistant strains. Early clinical signals are encouraging but indication-specific data are sparse: the DUOFAG Phase 1/2 trial (NCT06319235) is testing a topical anti–*S. aureus*/*P. aeruginosa* cocktail in SSIs, Armata's intravenous AP-SA02 cocktail reported a favorable Phase 2a result in complicated *S. aureus* bacteremia, and device-associated human evidence remains at the level of compassionate-use case series (Rubalskii et al., 2020). A 2025 systematic review confirms consistent staphylococcal antibiofilm activity in vitro and in vivo but stresses that cocktail composition, dosing, administration, and phage–antibiotic synergy must be standardized before routine use (Mobarezi et al., 2025). This R21 addresses that gap with three exploratory aims, each gated by an explicit go/no-go criterion. We will (1) design and characterize a matched lytic phage cocktail against a contemporary panel of *S. aureus* SSI/mesh isolates and quantify antibiofilm activity on clinically relevant mesh; (2) define phage–antibiotic synergy and resistance suppression on mesh-associated biofilm in vitro, then confirm benefit in a small foreign-body infection model; and (3) build and benchmark a rapid "phagogram" susceptibility pipeline to support future susceptibility-matched cocktail selection. The work is high-risk/high-reward and deliberately decision-focused: it generates the characterization, synergy, and matching data needed to justify an IND-enabling program for

implant-sparing *S. aureus* phage therapy, with the potential to convert mesh and prosthetic SSI from an implant-removal problem into a device-sparing, treatable one.

Specific Aims

Implanted mesh and graft infections caused by *S. aureus* are difficult to cure because biofilm on the device protects bacteria from antibiotics and host defenses, often forcing explantation. Lytic phages disrupt biofilm, kill persister cells, self-amplify locally, and act independently of antibiotic resistance, making them a rational device-sparing adjunct. However, indication-specific evidence is early — a registered SSI trial (DUOFAG, NCT06319235) and compassionate-use case series (Rubalskii et al., 2020) — and a 2025 systematic review emphasizes that cocktail design and phage–antibiotic synergy still require standardization (Mobarezi et al., 2025). Consistent with the exploratory R21 mechanism, we propose to generate the preclinical foundation and decision framework for matched, synergy-optimized anti–*S. aureus* phage therapy of mesh/graft SSI.

Aim 1. Design and characterize a matched lytic phage cocktail against contemporary *S. aureus* SSI/mesh isolates. Using a banked panel of clinical *S. aureus* SSI and device-associated isolates (including MRSA), we will determine host range of candidate lytic phages, select a complementary multi-phage cocktail to broaden lineage coverage and limit resistance, and quantify planktonic and biofilm-killing activity on standard hernia/vascular mesh coupons. Genome sequencing will confirm a strictly lytic lifecycle and absence of toxin, antibiotic-resistance, and lysogeny determinants. *Go/no-go:* a sequence-verified cocktail achieving the prespecified panel coverage and measurable on-mesh biofilm reduction [ILLUSTRATIVE: ≥80% panel coverage; ≥2-log biofilm CFU reduction].

Aim 2. Define phage–antibiotic synergy and resistance suppression on mesh-associated biofilm. We will test the Aim 1 cocktail combined with standard-of-care anti-staphylococcal antibiotics against mature mesh biofilm in vitro — measuring biofilm biomass, viable counts, persister reduction, and emergence of phage-resistant mutants — then evaluate the most promising combination in a small subcutaneous foreign-body (mesh) *S. aureus* infection model. *Go/no-go:* identification of ≥1 phage–antibiotic combination that is non-antagonistic and superior to antibiotic alone on mesh biofilm in vitro, advanced to a powered in vivo pilot.

Aim 3. Build and benchmark a rapid phagogram pipeline for cocktail matching. We will develop and validate a same-day susceptibility assay that predicts which cocktail components will be active against a patient's isolate, benchmarking turnaround and predictive accuracy against reference plaque/biofilm assays. *Go/no-go:* a same-day assay meeting prespecified agreement with reference methods [ILLUSTRATIVE: ≥85% categorical agreement; turnaround <8 h].

Impact: These exploratory studies will deliver a characterized, synergy-optimized cocktail and a rapid

matching assay — the evidence package required to advance implant-sparing *S. aureus* phage therapy toward an IND-enabling clinical study and, ultimately, to spare patients device explantation.

Significance

SSIs involving prosthetic implants are a leading source of surgical morbidity, and *S. aureus*, including MRSA, is a predominant cause. The biological obstacle is biofilm: once *S. aureus* colonizes mesh or graft material, the biofilm matrix renders the organism tolerant to antibiotics and shields it from host immunity, so durable cure commonly demands surgical removal of the device, with major cost, reoperation risk, and patient morbidity. There is therefore a high unmet need for a treatment that clears device-associated *S. aureus* biofilm **without** explantation — a need that falls squarely within NIAID's mission in antimicrobial resistance (AMR) and healthcare-associated infection.

Bacteriophages are mechanistically matched to this need (see Project Summary). The current evidence base, while early, is converging on this application. The DUOFAG Phase 1/2 trial (NCT06319235) is the first registered study aimed squarely at *S. aureus* SSI, applying a topical two-phage anti–*S. aureus*/*P. aeruginosa* cocktail to the wound. Systemic anti–*S. aureus* phage development is more advanced: AP-SA02 (Armata) reported a favorable Phase 2a result in complicated *S. aureus* bacteremia. For device/graft-associated SSI specifically, human data remain at the compassionate-use case-series level — most notably Rubalskii et al. (2020), an uncontrolled series in which 7 of 8 cardiothoracic patients with infections of vascular grafts, implanted devices, and surgical wounds (several involving *S. aureus*) achieved bacterial eradication when phages were added to antibiotics, with no severe adverse events. A 2025 systematic review confirms consistent staphylococcal antibiofilm efficacy in vitro and in vivo but explicitly identifies standardization of cocktail composition, dosing, administration, and phage–antibiotic synergy as the gap blocking routine clinical use (Mobarezi et al., 2025). This proposal targets that named gap directly, generating the matched-cocktail, synergy, and susceptibility-pipeline data NIAID's AMR program needs to de-risk a device-sparing phage therapeutic.

Innovation

This project is innovative in three respects. First, rather than treating phage therapy generically, it designs a cocktail specifically against a **contemporary panel of device-associated *S. aureus* SSI/mesh isolates** and measures activity on the actual mesh substrate, moving beyond planktonic assays toward the surface where clinical failure occurs. Second, it treats **phage–antibiotic synergy as a designed property rather than an afterthought**: we systematically test sub-inhibitory antibiotic pairing to enhance phage activity and suppress phage-resistant mutants on biofilm, directly answering the standardization gap named by the 2025 systematic review (Mobarezi et al., 2025). Third, it

develops a **rapid phagogram** to enable susceptibility-matched cocktail selection, prototyping the same-day workflow a surgeon would need to irrigate an infected implant with a matched cocktail at closure. Together these elements reframe mesh/graft SSI from an implant-removal problem toward an implant-sparing, treatable one, and lay groundwork for future engineered strategies (e.g., lysin-armed or phage-coated meshes) without depending on them for success.

Approach

Rigor and reproducibility (applies to all aims). All in vitro endpoints will be run in independent biological triplicate with technical replicates, using authenticated isolates and reference control strains. In vivo work will use predefined randomization, blinded outcome assessment, and a biostatistician-set sample size. Analyses are prespecified per aim (below); ambiguous or borderline go/no-go results trigger the stated alternatives rather than aim abandonment.

Aim 1 — Design and characterize a matched lytic phage cocktail against *S. aureus* SSI/mesh isolates

Rationale. Phage killing is strain-specific, so coverage across *S. aureus* lineages requires a rationally composed multi-phage cocktail; clinical use further demands strictly lytic phages free of resistance or virulence genes. Establishing host range and on-mesh antibiofilm activity is the prerequisite for all downstream work.

Experimental design. We will assemble a panel of clinical *S. aureus* SSI and device-associated isolates [ILLUSTRATIVE: ~40 isolates], including MRSA, characterized by susceptibility profiling and lineage typing. Candidate lytic anti–*S. aureus* phages from established collections will be screened for host range by spot and efficiency-of-plating (EOP) assays. A complementary subset [ILLUSTRATIVE: 3–4 phages] will be selected to maximize panel coverage and minimize shared resistance. Each phage will undergo whole-genome sequencing to confirm a lytic lifecycle and exclude known toxin, antibiotic-resistance, and lysogeny genes. Antibiofilm activity will be quantified on standard polypropylene hernia and vascular-graft mesh coupons using established biofilm assays (biomass staining, viable colony counts, confocal microscopy).

Analysis. Coverage reported as % of panel with EOP above threshold; biofilm activity as log10 CFU reduction versus untreated coupons (mixed-effects model across isolates/replicates).

Expected outcomes. A defined, sequence-verified cocktail covering the majority of the isolate panel [ILLUSTRATIVE: ≥80% coverage target] with measurable reduction of mesh-associated *S. aureus* biofilm, and a ranked component list for Aims 2–3.

Potential pitfalls & alternatives. If single-phage coverage is incomplete, we will expand the candidate pool and/or increase cocktail complexity; if a candidate carries undesirable genes, it will be excluded in favor of alternatives. If mesh biofilm proves highly variable, we will standardize inoculum and maturation time and anchor results to a reference biofilm assay.

Aim 2 — Define phage–antibiotic synergy and resistance suppression on mesh-associated biofilm

Rationale. Phages are usually combined with antibiotics in practice (as in Rubalskii et al., 2020), and sub-inhibitory antibiotics can enhance phage replication and suppress phage-resistant mutants. Because biofilm tolerance and resistance emergence are the central clinical failure modes, synergy must be characterized on the mesh surface and in vivo, not only in planktonic culture.

Experimental design. Against mature mesh biofilm formed by representative panel isolates, we will test the Aim 1 cocktail alone and combined with standard anti-staphylococcal antibiotics across a sub-inhibitory-to-therapeutic range, using checkerboard-style and time-kill designs. Endpoints: biofilm biomass, viable counts, persister-cell reduction, and frequency of phage-resistant mutants on serial passage. The most effective combination(s) will then be evaluated in a **small proof-of-concept** subcutaneous foreign-body (mesh implant) *S. aureus* infection model [ILLUSTRATIVE: small-animal model], comparing antibiotic alone, phage alone, and the combination on device and peri-implant bacterial burden. Consistent with the R21 scope, the in vivo study is a powered pilot to detect a combination effect, not a definitive efficacy trial.

Analysis. Synergy classified by accepted interaction criteria; in vivo bacterial burden compared across arms by prespecified test with multiplicity correction, biostatistician-set group sizes.

Expected outcomes. Quantitative identification of phage–antibiotic combinations that maximize biofilm clearance and suppress resistance on mesh, with in vivo confirmation that the combination reduces device-associated bacterial burden relative to antibiotic alone.

Potential pitfalls & alternatives. If antagonism is observed for a given antibiotic, we will test alternative agents and exposure sequencing (staggered vs. simultaneous). If the foreign-body model shows high variability, we will refine inoculum and adjust group size within the illustrative budget; if systemic dosing is confounding, we will emphasize local/topical application consistent with the SSI use case.

Aim 3 — Build and benchmark a rapid phagogram pipeline for cocktail matching

Rationale. Strain specificity means clinical deployment will likely require pretreatment susceptibility

("phagogram") matching. A same-day assay is needed for the envisioned workflow in which a surgeon matches and applies a cocktail at the time of operation.

Experimental design. We will adapt rapid susceptibility readouts (e.g., turbidity/metabolic and plaque-based indicators) into a streamlined phagogram that reports component-level activity against an isolate within a single working day [ILLUSTRATIVE: <8 h target]. Using the Aim 1 isolate panel as ground truth, we will benchmark the rapid assay's predictive accuracy and turnaround against reference plaque/EOP and biofilm assays, reporting sensitivity, specificity, and categorical agreement.

Analysis. Performance reported with confidence intervals against reference methods; turnaround logged per run.

Expected outcomes. A validated rapid phagogram with defined predictive performance and turnaround, suitable for incorporation into a future clinical protocol and for guiding cocktail selection.

Potential pitfalls & alternatives. If rapid readouts diverge from biofilm-based activity, we will calibrate thresholds and, if needed, retain a confirmatory reference assay; if same-day turnaround is not achievable, we will define the minimum feasible window and document the workflow trade-offs.

Timeline

[ILLUSTRATIVE] Two-year R21. **Months 1–9:** Aim 1 — isolate panel assembly, host-range screening, cocktail selection, genome verification, on-mesh antibiofilm assays (Aim 1 go/no-go at ~month 9). **Months 6–18:** Aim 2 — in vitro synergy and resistance studies, then foreign-body pilot (Aim 2 go/no-go before in vivo). **Months 10–24:** Aim 3 — rapid phagogram development and benchmarking. **Months 20–24:** data integration, IND-enabling gap analysis, and preparation of a follow-on translational proposal.

Budget Justification (modular)

[ILLUSTRATIVE] This R21 is budgeted modularly within the NIAID/NIH R21 cap of **$275,000 direct costs total over two years, with no more than $200,000 in any single year** (e.g., $150,000 in Year 1 and $125,000 in Year 2; final split set with institutional budget officers). **Personnel:** PI/PD (microbiology/phage biology) to lead design and oversight; Co-Investigator (surgical infectious disease/biofilm) for the mesh model and clinical relevance; one postdoctoral scientist (phage genomics/biofilm assays) and a part-effort research technician (isolate banking, host-range and phagogram assays). **Supplies:** clinical isolate handling, phage propagation and purification, mesh coupons, sequencing reagents, antibiotic-synergy consumables, and biofilm/microscopy reagents. **Animals:** per-diem and procedural costs for the subcutaneous foreign-body pilot (Aim 2). **Other:**

sequencing core services, biostatistics support for benchmarking analyses, and publication costs. No major equipment is requested. Final figures are placeholders to be set with institutional budget officers and kept within the R21 cap.

Vertebrate Animals

[ILLUSTRATIVE] Animal work is proposed in Aim 2 only: a small subcutaneous foreign-body (mesh implant) *S. aureus* infection model to test phage–antibiotic combinations on device-associated bacterial burden. The full Vertebrate Animals Section will justify species selection (a standard small-animal model appropriate for foreign-body infection), provide biostatistician-derived sample-size and statistical justification to minimize animal use while ensuring power, and describe randomization, blinded assessment, humane endpoints, analgesia, and veterinary oversight. IACUC approval will be obtained prior to any procedures; numbers and species will be finalized with the IACUC and biostatistics team.

Human Subjects / Clinical Trial

No human-subjects clinical trial is conducted within this R21; the work is preclinical and uses banked, de-identified clinical bacterial isolates, the use of which will be reviewed by the IRB/biosafety committee for human-derived materials. The proposal is explicitly designed to enable a future first-in-indication clinical study. For investigational phage administration to patients, that future study would proceed under FDA oversight via an investigational new drug (IND) submission — including the emergency/expanded-access IND (eIND) route that has supported prior compassionate phage use for device-associated infection (Rubalskii et al., 2020) — with full IRB approval, informed consent, and trial registration. This R21 will generate the characterization, synergy, and susceptibility-matching data needed to support that IND-enabling step, complementing registered efforts such as DUOFAG (NCT06319235).

Team & Environment

[Template — fill with real names/institutions.] **Contact PD/PI — [Name, PhD], Phage Biology/Microbiology, [Institution]:** cocktail design, host-range and genomic characterization, overall direction. **Co-I — [Name, MD], Surgical Infectious Diseases, [Institution]:** mesh-infection relevance, foreign-body model, translational/IND strategy. **Co-I — [Name, PhD], Biofilm/Antimicrobial Pharmacology:** phage–antibiotic synergy and resistance analyses. **Collaborator — Clinical Microbiology Laboratory, [Institution]:** isolate banking and phagogram validation. **Collaborator — [Name], Regulatory Affairs:** IND/eIND pathway guidance. **Consultant**

— Biostatistics Core: design, power, and benchmarking analyses. **Environment:** the host institution provides BSL-2 microbiology and phage-production facilities, genomics and microscopy cores, an AAALAC-accredited animal facility with IACUC oversight, and established IRB and institutional biosafety committees. Where feasible, the team will engage established phage centers and compassionate-use phage-bank programs experienced in device infections to inform cocktail sourcing and the translational pathway.

References

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