A Defined Dual-Target Bacteriophage Cocktail for *Staphylococcus aureus* and *Pseudomonas aeruginosa* Biofilm Infection in Diabetic Foot Ulcers: From Mechanism to IND-Readiness

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Project Summary / Abstract

Diabetic foot ulcers (DFUs) are a leading cause of non-traumatic lower-limb amputation and one of the most costly complications of diabetes. These chronic wounds are frequently dominated by *Staphylococcus aureus* and *Pseudomonas aeruginosa*, which coexist as antibiotic-tolerant, often polymicrobial biofilms that systemic antibiotics penetrate poorly. Rising methicillin-resistant *S. aureus* (MRSA) and multidrug-resistant *P. aeruginosa*, compounded by the impaired perfusion and immune dysfunction intrinsic to diabetes, render many infections refractory to standard care and drive progression to osteomyelitis and amputation.

Lytic bacteriophages are mechanistically matched to this problem: applied **topically** at the wound bed, they bypass the perfusion barrier that limits systemic antibiotic delivery to ischemic tissue, self-amplify on their bacterial host, carry depolymerases that degrade biofilm matrix, and kill in a strain-specific manner that spares commensal flora and healing tissue. A defined multi-phage cocktail can cover both *S. aureus* and *P. aeruginosa* simultaneously and raise the genetic barrier to phage resistance relative to single phages. Early-phase clinical data are encouraging but preliminary: the TP-102 cocktail (targeting *S. aureus*, *P. aeruginosa*, and/or *Acinetobacter baumannii*) was safe and well tolerated in the randomized, double-blind REVERSE Phase 1/2a DFU study, which was explicitly underpowered for efficacy (Nir-Paz et al., 2025; NCT04803708). A 2025 systematic review concluded that the DFU phage evidence base — 21 studies through 2024 — remains limited to small-scale work and calls for rigorous trials, standardized protocols, and broader cocktails for polymicrobial infection (Esfandiari et al., 2025).

This proposal supplies the missing mechanistic and translational foundation. We will (**Aim 1**) define

the host range and biofilm-disrupting activity of a defined dual-target *S. aureus*/*P. aeruginosa* cocktail against a contemporary DFU clinical-isolate panel; (**Aim 2**) determine phage pharmacodynamics, phage–antibiotic interactions, and resistance dynamics in a diabetic wound-infection model with prospective resistance surveillance; and (**Aim 3**) establish a rapid susceptibility-matching ("phagogram") assay and a manufacturing/regulatory framework to enable an investigational new drug (IND) application. The expected outcome is a mechanistically grounded, manufacturable, susceptibility-matched cocktail and an IND-ready package positioning topical phage therapy as a near-term route to reduce drug-resistant DFU amputations — directly advancing NIDDK's mission to reduce the burden of diabetes complications.

Specific Aims

DFUs driven by *S. aureus* and *P. aeruginosa* biofilms are a major cause of amputation, and standard antibiotics are limited by poor biofilm penetration, impaired perfusion in diabetic tissue, and rising resistance. Topical lytic bacteriophage cocktails self-amplify, carry biofilm-degrading depolymerases, and kill strain-specifically. They have shown safety in a randomized DFU trial, but that trial was underpowered for efficacy, and the broader evidence base lacks the mechanistic pharmacodynamics, resistance characterization, and manufacturable, susceptibility-matched product needed to support a pivotal program (Nir-Paz et al., 2025; Esfandiari et al., 2025). **Central hypothesis:** a rationally composed dual-target cocktail, paired with synergistic short antibiotic courses, will reduce *S. aureus*/*P. aeruginosa* biofilm bioburden and improve wound closure in a diabetic wound model while constraining resistance, and can be matched to patient isolates and manufactured to IND-enabling specifications.

Aim 1 — Define the host range and biofilm-disrupting activity of a dual-target *S. aureus*/*P. aeruginosa* phage cocktail against a contemporary DFU clinical-isolate panel. We will assemble an IRB-approved biobank of *S. aureus* (including MRSA) and *P. aeruginosa* (including MDR) DFU isolates and characterize candidate lytic phages — prioritizing broad-host-range Kayvirus/Silviavirus-type *S. aureus* phages and depolymerase-bearing anti-*Pseudomonas* phages — for host range (efficiency of plating), lytic kinetics, and single- and dual-species biofilm reduction. We will formulate a cocktail optimized for coverage breadth and suppression of resistant mutants. *Go/no-go milestone:* cocktail covers ≥[ILLUSTRATIVE] 80% of panel isolates per species with ≥[ILLUSTRATIVE] 2-log dual-species biofilm reduction.

Aim 2 — Determine phage pharmacodynamics, phage–antibiotic interactions, and resistance dynamics in a diabetic wound-infection model. In a diabetic rodent wound model infected with an established *S. aureus*/*P. aeruginosa* biofilm, we will quantify topical phage dosing, bioburden

reduction, and wound closure against vehicle, and systematically classify phage–antibiotic combinations as synergistic, additive, or antagonistic. Longitudinal resistance surveillance will test whether cocktail composition and adjunctive short antibiotic courses constrain phage-resistant escape. *Go/no-go milestone:* ≥[ILLUSTRATIVE] 2-log bioburden reduction versus vehicle and an identified non-antagonistic phage–antibiotic pairing.

Aim 3 — Establish a susceptibility-matching assay and a manufacturing/regulatory framework to enable an IND. We will develop and validate a rapid, clinically deployable phage-susceptibility ("phagogram") assay matching a patient's isolates to cocktail components, and define purification, endotoxin, potency, stability, and lot-release specifications aligned with FDA expectations for investigational phage. We will assemble an IND-enabling package and define both a conventional IND pathway for the defined cocktail and the emergency/expanded-access IND (eIND) route for individualized use. *Go/no-go milestone:* validated same-week phagogram (concordance with reference host-range data ≥[ILLUSTRATIVE] 90%) and a cocktail lot meeting pre-specified endotoxin/potency/stability criteria.

Impact. By delivering a mechanistically validated, manufacturable, susceptibility-matched cocktail and an IND-ready package, this work provides the translational foundation to move topical phage therapy toward a licensed biologic that could meaningfully reduce amputations following drug-resistant DFUs.

Significance

The problem is central to NIDDK's mission. DFUs are among the most consequential complications of diabetes and a leading cause of non-traumatic lower-limb amputation, carrying high mortality, disability, and cost. Reducing the burden of diabetes complications — including the chronic, non-healing wounds that lead to amputation — is squarely within NIDDK's purview.

The microbiology is distinctive and difficult. *S. aureus* and *P. aeruginosa* dominate DFU infection and frequently coexist as polymicrobial, antibiotic-tolerant biofilms embedded in an exopolysaccharide matrix that systemic antibiotics penetrate poorly. Rising MRSA and MDR *P. aeruginosa*, together with the impaired perfusion and immune dysfunction intrinsic to diabetes, make many infections refractory to standard wound care, and progression to osteomyelitis is a frequent precursor to amputation (Esfandiari et al., 2025; Plumet et al., 2025).

Bacteriophages address several failure modes of conventional therapy at once. Because they are applied topically into the wound bed, they bypass the perfusion barrier that limits systemic antibiotic delivery to ischemic diabetic tissue. Because lytic phages self-amplify on their host, dosing is partly

self-sustaining where bacteria are present. Anti-*Pseudomonas* phages contribute depolymerases that strip exopolysaccharide and expose embedded cells, directly attacking the biofilm matrix that protects DFU pathogens. And because phage killing is strain-specific, the approach spares commensal flora and wound-healing tissue rather than indiscriminately damaging the wound microenvironment. A 2025 review of phage therapy for *S. aureus* diabetic foot infection synthesizes preclinical and clinical evidence of bacterial-load reduction and improved wound healing, with no major adverse effects, and underscores the rationale for this approach (Plumet et al., 2025).

The clinical signal is real but preliminary, and the gap is well defined. The TP-102 cocktail (targeting *S. aureus*, *P. aeruginosa*, and/or *A. baumannii*) was safe and well tolerated in the randomized, double-blind REVERSE Phase 1/2a study in infected and non-infected DFUs; numerical trends favored TP-102 (microbiological reduction 80% vs 50%; 50% wound closure 71.4% vs 33.3%), but the study was explicitly **underpowered** to demonstrate superiority and the differences were not statistically significant (Nir-Paz et al., 2025; NCT04803708). The associated BX211 program advanced to a Phase 2 diabetic foot osteomyelitis study (DANCE) reported in abstract form (NCT04803708 record / Open Forum Infect Dis. 2026). A 2025 systematic review of 21 studies concluded that the DFU phage evidence base is promising but limited to small-scale work, calling for randomized controlled trials, standardized protocols, broader cocktails for polymicrobial infection, and defined regulatory pathways — with no licensed product to date (Esfandiari et al., 2025). The field therefore needs rigorous mechanistic pharmacodynamics, resistance characterization, and a manufacturable, susceptibility-matched product — **precisely the gap this proposal fills.**

Innovation

This proposal is innovative in four respects.

1. **A defined dual-target cocktail.** Rather than a single-pathogen product, we advance a cocktail purpose-built to cover both dominant DFU pathogens (*S. aureus* and *P. aeruginosa*) simultaneously, addressing the polymicrobial reality that recent reviews identify as a key gap (Esfandiari et al., 2025).

2. **Phage–antibiotic interaction treated as a measured variable.** Because synergy between phages and sub-lethal antibiotics is mechanistically plausible but not universal across agents, we will empirically map combinations that help versus those that do not, to rationally pair phage with the short antibiotic courses used clinically — rather than assuming synergy.

3. **Prospective, longitudinal resistance surveillance embedded in vivo.** We build resistance monitoring directly into a diabetic wound model to test whether cocktail design and

depolymerase activity constrain phage-resistant escape, generating the resistance-dynamics data the field currently lacks.

4. **Biology coupled to a deployable susceptibility-matching assay and a manufacturing/regulatory package.** We pair the cocktail with a rapid "phagogram" and an IND-enabling CMC/regulatory framework, directly enabling the personalized "swab-to-tailored-cocktail" workflow that distinguishes phage therapy from fixed-formulation drugs.

We deliberately rely on **natural lytic phages**, matching the clinical-stage state of the art (Nir-Paz et al., 2025); engineered or CRISPR-armed phages are noted only as a future direction, not part of this plan.

Approach

Rigor and reproducibility (applies to all Aims). All quantitative endpoints will be pre-specified with biological replicates and powered group sizes [ILLUSTRATIVE]; in vivo studies will use randomization, blinded outcome assessment, and pre-registered analysis plans. **Sex as a biological variable:** both sexes will be included in Aim 2, powered to detect sex-based differences in bioburden and closure [ILLUSTRATIVE]. Phage stocks will be genome-sequenced to confirm lytic lifestyle and absence of toxin/resistance genes. Statistical analyses will be developed with the biostatistician and reported with effect sizes and confidence intervals.

Aim 1 — Host range and biofilm disruption of a dual-target cocktail

Rationale. Cocktail efficacy depends on covering the genetic diversity of clinical *S. aureus* and *P. aeruginosa* and on disrupting the protective biofilm matrix. Broad-host-range Kayvirus/Silviavirus-type *S. aureus* phages combine wide coverage with strong biofilm activity, and depolymerase-bearing anti-*Pseudomonas* phages expose matrix-embedded cells (Plumet et al., 2025).

Experimental design. We will assemble a DFU clinical-isolate biobank (*S. aureus* including MRSA; *P. aeruginosa* including MDR) under IRB-approved collection with de-identified metadata. Candidate lytic phages will be screened by spot and efficiency-of-plating assays for host range and by liquid culture for lytic kinetics. Biofilm activity will be assessed in single-species and *S. aureus*/*P. aeruginosa* dual-species biofilms using biomass (crystal violet) and viable-count endpoints, with depolymerase activity scored phenotypically (halo formation). Candidates will be combined into a cocktail and optimized in vitro for coverage breadth and suppression of resistant mutants (time-kill and mutant-frequency assays).

Expected outcomes. A defined cocktail covering ≥[ILLUSTRATIVE] 80% of the contemporary DFU isolate panel per species, with demonstrable single- and dual-species biofilm reduction (≥[ILLUSTRATIVE] 2-log viable-count drop) and reduced emergence of resistant mutants relative to single phages.

Potential pitfalls & alternatives. If coverage gaps remain for particular lineages, we will expand phage candidates or add components; if dual-species biofilm proves more refractory than single-species, we will incorporate brief debridement-mimicking disruption or sequence phage application by species.

Aim 2 — Pharmacodynamics, phage–antibiotic interaction, and resistance dynamics in vivo

Rationale. Translation requires knowing how topical phage behaves in a diabetic wound: effective dose, bioburden reduction, wound closure, and whether adjunctive antibiotics help or hinder. Because synergy is real but not universal, it must be measured rather than assumed.

Experimental design. In a diabetic rodent wound-infection model colonized with an established *S. aureus*/*P. aeruginosa* biofilm, we will test topical cocktail dosing regimens against vehicle control, with primary endpoints of wound bioburden (viable counts) and wound-area closure over time, plus wound-bed histology. In parallel, the cocktail will be combined with clinically relevant antibiotics across a matrix of timings and doses to classify interactions as synergistic, additive, or antagonistic, explicitly including agents where synergy may be absent. Longitudinal resistance surveillance will recover bacteria across time to quantify phage-resistant variants and susceptibility shifts across cocktail and cocktail-plus-antibiotic arms.

Expected outcomes. A defined effective topical dosing regimen; quantitative evidence of biofilm bioburden reduction (≥[ILLUSTRATIVE] 2-log vs vehicle) and improved closure; an interaction map identifying beneficial phage–antibiotic pairings; and evidence on whether cocktail design plus short antibiotic courses constrains resistance.

Potential pitfalls & alternatives. If single-dose phage is rapidly cleared, we will use repeat dosing or sustained-release dressings; if antagonism dominates for a given antibiotic, we will exclude it and prioritize synergistic or neutral pairings; if the model under-recapitulates chronic biofilm, we will extend infection establishment before treatment.

Aim 3 — Susceptibility matching and manufacturing/regulatory framework

Rationale. Personalized phage therapy needs a rapid way to confirm a patient's isolates are covered by the cocktail, plus a defined, quality-controlled product to support regulatory review and the

absence of any licensed DFU phage to date (Esfandiari et al., 2025).

Experimental design. We will develop and validate a rapid phage-susceptibility ("phagogram") assay against the isolate biobank, benchmarking turnaround and concordance with reference host-range data toward a same-week match. In parallel, we will define product specifications: purification and endotoxin reduction, titer/potency assays, stability and shelf-life under storage, and lot-release quality control. We will assemble these into an IND-enabling package and define the FDA pathway for investigational phage — both a conventional IND for a defined-cocktail study and, separately, the emergency/expanded-access IND (eIND) route used for individualized phage treatment under IRB oversight.

Expected outcomes. A validated, clinically deployable susceptibility assay (concordance ≥[ILLUSTRATIVE] 90%; same-week turnaround) and a documented CMC/QC and regulatory package suitable to support an IND (and eIND) and a future clinical study.

Potential pitfalls & alternatives. If assay turnaround is too slow for clinical use, we will streamline readouts (e.g., metabolic or luminescent endpoints); if endotoxin or stability specifications are not met, we will refine purification and formulation before locking the cocktail.

Timeline

[ILLUSTRATIVE] **Years 1–2:** Aim 1 isolate biobank, phage characterization, and cocktail formulation. [ILLUSTRATIVE] **Years 2–4:** Aim 2 in vivo pharmacodynamics, phage–antibiotic interaction mapping, and resistance surveillance. [ILLUSTRATIVE] **Years 3–5:** Aim 3 susceptibility-assay validation and manufacturing/regulatory package, with an FDA pre-IND interaction in [ILLUSTRATIVE] Year 4 and IND-enabling activities completing in [ILLUSTRATIVE] Year 5. Aims overlap to de-risk cocktail composition before locking manufacturing specifications; each transition is gated by the Aim's go/no-go milestone.

Budget Justification

Modular R01 (figures illustrative only). [ILLUSTRATIVE] ~$250,000 direct costs per year for [ILLUSTRATIVE] 5 years. **Personnel:** [ILLUSTRATIVE] PI (microbiology/phage biology) at meaningful effort; [ILLUSTRATIVE] co-investigators in infectious diseases/podiatry and in regulatory/manufacturing; [ILLUSTRATIVE] postdoctoral scientists; [ILLUSTRATIVE] research technicians; and a [ILLUSTRATIVE] part-time biostatistician. **Other costs:** clinical-isolate

biobanking and sequencing; phage characterization and biofilm assays; diabetic wound-model animal costs and histology (Aim 2); susceptibility-assay validation and product QC/stability (Aim 3); and publication/dissemination. Equipment is assumed largely available through institutional cores; itemized direct costs and indirect costs to be calculated per the awardee institution's negotiated rate.

Vertebrate Animals

Animal work is proposed in Aim 2 using a diabetic rodent wound-infection model to evaluate topical phage pharmacodynamics, bioburden reduction, wound closure, phage–antibiotic interaction, and resistance dynamics. A full Vertebrate Animals Section — species/strain justification, group sizes by power analysis [ILLUSTRATIVE], inclusion of both sexes [ILLUSTRATIVE], wound-infection and topical-dosing procedures, humane endpoints, anesthesia/analgesia, and euthanasia consistent with AVMA guidelines — will be provided and approved by the institutional IACUC before any animal work begins. Numbers of animals will be minimized consistent with statistical rigor (the 3Rs), and outcome assessment will be blinded.

Human Subjects / Clinical Trial

No interventional clinical trial is proposed in this R01. Human involvement is limited to collection and use of DFU bacterial isolates and associated de-identified clinical data for the biobank (Aims 1 and 3) under IRB approval with applicable informed-consent/HIPAA provisions; this is laboratory research on bacterial isolates and does not meet the NIH definition of a clinical trial. The proposal explicitly prepares the regulatory route for future clinical use of investigational phage: because phage products are biologics, clinical administration requires FDA oversight. A defined-cocktail study would proceed under a conventional IND, while individualized therapy has been enabled separately through the emergency/expanded-access IND (eIND) mechanism with IRB oversight; the two pathways are complementary, not interchangeable. The randomized DFU phage study that motivates this work was conducted under its respective regulatory and ethics framework (Nir-Paz et al., 2025; NCT04803708).

Investigators & Environment

This program requires an interdisciplinary US-based team (template roles to be filled with named

investigators and institutions [ILLUSTRATIVE]): a **Principal Investigator** with phage biology/microbiology expertise; a **Co-Investigator in Infectious Diseases** and a **Co-Investigator in Podiatric/Wound Medicine** providing DFU clinical isolates and clinical context; a **Vertebrate Animal / Wound-Model Lead** for Aim 2; a **Phage Manufacturing & Regulatory Lead** (CMC, endotoxin/QC, IND/eIND strategy); a **Biostatistician**; and a **Clinical Microbiology Laboratory Director** to host the susceptibility ("phagogram") assay. The environment must include a clinical microbiology laboratory and isolate biobank, phage characterization and biofilm facilities, an AAALAC-accredited animal facility supporting a diabetic wound model, and institutional regulatory (IRB/IACUC) and CMC support. The team's combined microbiology, wound-care, regulatory, and biostatistical expertise is matched to the aims and to the gaps identified in the current DFU phage literature (Esfandiari et al., 2025; Plumet et al., 2025). Complementary US funders for related aims include NIGMS and the DoD CDMRP.

References

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