% **Funder/Mechanism:** NINR · R21 (Exploratory/Developmental Research Grant; clinical-trial-optional). [ILLUSTRATIVE budget/scope markers retained throughout.]

Personalized Phage Cocktails Targeting Polymicrobial Biofilms in Chronic Venous Leg and Pressure Ulcers: An Exploratory Proof-of-Concept Study

Project Summary / Abstract

Chronic venous leg ulcers (VLUs) and pressure ulcers afflict millions of older and immobilized Americans and frequently fail to heal because their wound beds harbor dense polymicrobial biofilms — matrix-encased bacterial communities commonly led by *Pseudomonas aeruginosa* and *Staphylococcus aureus* (often MRSA), with *Enterococcus*, *Klebsiella*, and *Escherichia coli*. Biofilm-resident bacteria are widely recognized to tolerate antibiotics far above the concentrations that kill the same organisms growing planktonically, so systemic antibiotics penetrate the wound bed poorly, fail to clear colonization, and exert selective pressure for resistance while the wound stays open. Lytic bacteriophages are a mechanistically distinct, topically applied complement: they self-amplify wherever susceptible bacteria persist, many encode depolymerases that degrade the biofilm matrix, they kill antibiotic-resistant strains by a route orthogonal to antibiotics, and they can be combined into cocktails spanning the several pathogens of a single ulcer. Indication-specific human evidence is early but encouraging: an FDA-cleared Phase I safety trial of an 8-phage cocktail (WPP-201) in 42 VLU patients reported no product-attributable adverse events (Rhoads 2009); a biodegradable matrix co-delivering phages and an antibiotic improved outcomes in infected venous stasis ulcers (Markoishvili 2002); and a 2024 randomized, double-blind RCT of customized phage cocktails in 60 chronic-wound patients reported 93.3% wound sterility at a median of 39 days and complete healing by 90 days versus persistent colonization on placebo (Karn 2024). A Phase 1/2 trial of a phage product (BACTELIDE) in colonized pressure injuries is registered (NCT04815798). Yet no adequately powered US efficacy trial has reported and no phage product is licensed for these indications. This exploratory R21 will establish biofilm-targeted proof of concept by: (1) profiling the polymicrobial communities of VLUs and pressure ulcers and assembling a coverage-matched, depolymerase-enriched lytic-phage cocktail validated against patient-derived isolates; and (2) quantifying cocktail-

mediated biofilm disruption and phage–antibiotic synergy in patient-derived single- and mixed-species biofilms in vitro and in one established preclinical wound-biofilm model. A focused regulatory and clinical-readiness package (target product profile, IND pre-submission, and a powered Phase II design) positions the matched-cocktail approach for first-in-US clinical evaluation. The work is high-impact and tightly aligned with NINR's chronic wound-care and symptom-science mission.

Specific Aims

Stalled healing in chronic VLUs and pressure ulcers is driven substantially by polymicrobial biofilms, in which bacteria are broadly recognized to tolerate antibiotics far above planktonic killing concentrations, leaving wounds colonized for months. Bacteriophages offer an orthogonal, topically applied, self-amplifying, biofilm-degrading complement; recent randomized human data in chronic wounds are promising (Karn 2024; Rhoads 2009), but US proof of concept against the *mixed-species* biofilms specific to these wounds is lacking. This exploratory R21 is deliberately scoped to two laboratory aims that generate the rigorous preclinical and regulatory package required to justify a future powered clinical trial; no human or animal intervention is performed under this award.

Aim 1. Profile ulcer biofilm communities and assemble a coverage-matched, anti-biofilm phage cocktail. From archived/consented VLU and pressure-ulcer wound-bed specimens, we will isolate and characterize the dominant culturable pathogens (*P. aeruginosa*, *S. aureus*/MRSA, *Enterococcus*, *Klebsiella*, *E. coli*) with antibiotic-resistance phenotypes, and screen candidate lytic phages by host-range and plaque assay, prioritizing depolymerase/matrix-degrading phages. *Deliverable:* a coverage-mapped, defined cocktail with validated lytic activity across the prevalent pathogens, plus a characterized isolate biobank. *Go/No-Go:* a candidate cocktail covering ≥80% of isolates of each of the two most prevalent species [ILLUSTRATIVE threshold].

Aim 2. Quantify biofilm disruption and phage–antibiotic synergy preclinically. Using patient-derived single- and mixed-species biofilms in vitro and one established preclinical wound-biofilm model, we will measure cocktail-mediated reductions in viable biofilm burden and matrix, test whether sublethal antibiotic exposure potentiates phage replication (phage–antibiotic synergy), and screen for phage-driven resensitization of resistant isolates. *Deliverable:* quantitative anti-biofilm effect sizes plus defined dose, timing, and companion-antibiotic parameters. *Go/No-Go:* ≥2-$\log_{10}$ reduction in mixed-species biofilm viable burden versus untreated control [ILLUSTRATIVE].

Clinical-translation readiness (cross-cutting). Aim deliverables feed a target product profile, an FDA IND pre-submission (pre-IND) meeting package for an investigational topical phage cocktail, and a statistically powered Phase II design — positioning the approach for first-in-US clinical evaluation under a subsequent award.

Impact. Demonstrating that a coverage-matched, depolymerase-enriched cocktail measurably disrupts the *polymicrobial* biofilms of these specific wounds would de-risk a non-antibiotic path to closing ulcers that otherwise stay open for months — directly advancing NINR's chronic wound-care mission.

Significance

VLUs and pressure ulcers are among the most common chronic wounds in nursing and long-term-care practice, disproportionately burdening older, immobilized, and medically complex Americans — populations central to NINR's mission. Their defining clinical problem is stalled healing, and a principal biological driver is the polymicrobial biofilm: a dense, matrix-encased community typically led by *P. aeruginosa* and *S. aureus* (frequently MRSA) alongside *Enterococcus*, *Klebsiella*, and *E. coli*. Because biofilm-resident cells are broadly recognized to tolerate antibiotics far above the concentrations that kill their planktonic counterparts, systemic antibiotics penetrate poorly and fail to sterilize the wound bed while exerting selective pressure that fosters multidrug resistance. The result is a self-perpetuating cycle of colonization, inflammation, and non-healing that current standard of care does not reliably break.

Bacteriophages are mechanistically well-matched to this problem. Applied topically onto the wound bed, lytic phages self-amplify wherever susceptible host bacteria are present, so the delivered dose tracks the infection. Many wound phages carry depolymerases that degrade the exopolysaccharide matrix, breaching the barrier that shields embedded and persister cells; progeny phages then propagate through the biofilm. Critically, phages kill antibiotic-resistant strains by a mechanism orthogonal to antibiotics, can resensitize bacteria to drugs, and can be assembled into cocktails spanning the several pathogens within a single ulcer — precisely the coverage a polymicrobial wound demands.

Indication-specific human evidence, while early, is directly on target. An FDA-cleared Phase I randomized, double-blind, controlled safety trial of the 8-phage WPP-201 cocktail — formulated against *P. aeruginosa*, *S. aureus*, and *E. coli* — in 42 chronic VLU patients reported no product-attributable adverse events (Rhoads 2009). A sustained-release biodegradable poly(ester amide) matrix co-impregnated with bacteriophages and an antibiotic showed promise in the management of infected venous stasis and other poorly healing ulcers (Markoishvili 2002). A 2024 randomized, placebo-controlled, double-blind RCT of customized phage cocktails in chronic wounds (n=60; 30 per arm), including MDR and biofilm-associated infections, reported 93.3% wound sterility at a median of 39 days and complete healing by 90 days, versus 83.3% of placebo wounds remaining colonized without healing (Karn 2024). For pressure ulcers specifically, a Phase 1/2 trial of a phage product (BACTELIDE) added to standard of care in *S. aureus*-, *P. aeruginosa*-, and *K. pneumoniae*-colonized pressure injuries is registered (NCT04815798).

Two gaps remain. First, these encouraging studies largely treated wounds as single-pathogen targets or used fixed cocktails; none rigorously mapped cocktail coverage onto the *mixed-species* biofilm communities that define US VLUs and pressure ulcers, nor quantified mixed-species biofilm disruption with defined dose/synergy parameters. Second, no licensed US product and no adequately powered US efficacy trial exists. This R21 supplies exactly the missing biofilm-targeted, coverage-matched preclinical and regulatory-readiness package needed to advance the field.

Innovation

This proposal is innovative in three respects. **First**, it reframes phage therapy around the *polymicrobial biofilm* as the explicit therapeutic target rather than a single planktonic pathogen, prioritizing depolymerase/matrix-degrading phages and validating coverage against the mixed-species communities actually recovered from VLU and pressure-ulcer beds. **Second**, it experimentally interrogates phage–antibiotic synergy and phage-driven resensitization as deliberate, harnessed mechanisms — using phages to make recalcitrant, frequently MDR biofilms more susceptible, rather than treating phages as standalone agents. **Third**, it pairs the science with a concrete US regulatory-readiness deliverable (target product profile + pre-IND package + powered Phase II design), bridging from the largely compassionate/magistral use seen to date toward a licensable, evidence-based path. Throughout, the work stays grounded in sustained-release/co-delivery concepts already shown compatible with wound application (Markoishvili 2002), rather than speculative engineered constructs that remain preclinical.

Approach

Overview and rigor. Two laboratory aims generate the proof-of-concept and parameter set; no intervention in humans or animals is performed under this award. Experiments use predefined, quantitative endpoints with explicit Go/No-Go criteria, biological replication, blinded enumeration where feasible, and authenticated key resources (see *Rigor & Reproducibility*). Bacterial sex is not applicable; for human-derived specimens, donor sex will be recorded and isolate panels balanced across donor sex where feasible (see *Human Specimens / SABV*).

Aim 1 — Profile ulcer biofilm communities and assemble a coverage-matched anti-biofilm phage cocktail

Rationale. Coverage of a polymicrobial ulcer requires combining multiple lytic phages spanning its dominant pathogens. Because lytic phages bind strain-specific surface receptors (LPS, teichoic acids, capsule, pili), cocktail composition must be matched to patient-derived isolates, and depolymerase-bearing phages are prioritized for matrix-degrading activity. Critically, no existing fixed cocktail

covers the full species panel — e.g., WPP-201 targets only *P. aeruginosa*, *S. aureus*, and *E. coli* (Rhoads 2009) and does not address *Enterococcus* or *Klebsiella* — motivating an empirically matched design.

Experimental design. From archived/biobanked or prospectively consented VLU and pressure-ulcer wound-bed specimens, we will isolate the dominant culturable species (*P. aeruginosa*, *S. aureus*/MRSA, *Enterococcus*, *Klebsiella*, *E. coli*) and record antibiotic-susceptibility phenotypes. Candidate lytic phages — drawn from established collections and component phages of previously studied wound cocktails — will be screened against the isolate panel by quantitative host-range (efficiency-of-plating) and plaque assays, with explicit preference for phages exhibiting depolymerase/matrix-degrading halos. A defined multi-phage cocktail will be assembled to maximize cross-species coverage; where single-species coverage gaps persist, a personalized/customized formulation strategy analogous to Karn 2024 will be adopted. [ILLUSTRATIVE: ~30 donor specimens; ~8–12 phages screened; triplicate EOP determinations.]

Expected outcomes. A coverage-mapped, defined cocktail with validated lytic activity against the prevalent VLU/pressure-ulcer pathogens, and a characterized isolate biobank for Aim 2.

Potential pitfalls & alternatives. *(i) Phage-resistant or poorly covered isolates* — broaden the candidate phage panel and adopt personalized cocktails for coverage gaps. *(ii) Specimen access slower than projected* — leverage existing wound-isolate biobanks and reference strains to maintain the screening pipeline. *(iii) Depolymerase activity not visually evident* — supplement halo screening with genomic depolymerase annotation and matrix-degradation assays in Aim 2.

Aim 2 — Quantify biofilm disruption and phage–antibiotic synergy preclinically

Rationale. Biofilm antibiotic tolerance is the core obstacle. Demonstrating that the matched cocktail reduces viable mixed-species biofilm burden — and that sublethal antibiotics potentiate phage replication (phage–antibiotic synergy, PAS) — establishes the mechanism, dose, and combination parameters required for clinical translation.

Experimental design. Using single- and mixed-species biofilms grown from Aim 1 isolates in vitro and in one established preclinical wound-biofilm model, we will quantify viable counts (CFU/biofilm) and matrix/biomass after cocktail exposure versus untreated and antibiotic-only controls. PAS will be tested by combining the cocktail with sublethal concentrations of clinically relevant antibiotics; surviving isolates will be screened for restored antibiotic susceptibility (resensitization). Dose-ranging and exposure timing will inform a candidate clinical regimen. [ILLUSTRATIVE: ≥3 biological replicates; effect-size and Go/No-Go thresholds predefined; blinded CFU enumeration.]

Expected outcomes. Quantitative mixed-species biofilm-burden reductions, identification of synergistic phage–antibiotic combinations, and documented resensitization events — defining dose, timing, and companion-antibiotic choices for a future trial.

Potential pitfalls & alternatives. *(i) Emergent phage resistance under selection* — cocktail multiplicity and PAS combinations are designed to suppress resistance; cocktail membership will be iterated. *(ii) In vitro model under-predicts in vivo behavior* — a single, well-validated preclinical wound-biofilm model is included as the bridging system; if an animal wound-biofilm study is later judged necessary, it will be conducted under a *separate* future award rather than within this R21 (see *Vertebrate Animals*). *(iii) Mixed-species cultures destabilize in coculture* — use defined dual/triple-species ratios and validated coculture conditions, reporting per-species CFU.

Timeline

[ILLUSTRATIVE] Two-year R21. **Months 1–10:** Aim 1 — isolate characterization, phage screening, and cocktail assembly; begin target product profile. **Months 8–22:** Aim 2 — in vitro and wound-model biofilm and PAS studies (overlapping Aim 1). **Months 18–24:** data synthesis, FDA pre-IND package, and powered Phase II design. No human or animal intervention occurs within this award.

Budget Justification (modular)

[ILLUSTRATIVE] Requested within the standard R21 ceiling of [ILLUSTRATIVE: $275,000 direct costs] over [ILLUSTRATIVE: 2 years], in a [ILLUSTRATIVE: $200,000 / $75,000] modular split (or as allowed). **Personnel:** PI effort to direct phage and wound-microbiology work and a microbiologist/research technician for isolate, biofilm, and PAS assays ([ILLUSTRATIVE: ~1.0–1.5 FTE total]); regulatory/biostatistics support is engaged on a consultative basis for the pre-IND and Phase II design deliverables. **Supplies:** bacterial culture and phage-propagation reagents; biofilm and wound-model consumables; antibiotic-susceptibility and depolymerase/molecular assays. **Other:** FDA pre-IND preparation/regulatory consultation, statistical consultation, and specimen-handling costs. No major equipment is requested; existing institutional BSL-2 microbiology and wound-care infrastructure is assumed. (No clinical-trial or human-intervention costs are included, consistent with the laboratory scope.)

Rigor & Reproducibility

Endpoints are quantitative and predefined with explicit Go/No-Go thresholds; experiments use ≥3 biological replicates with appropriate controls (untreated, antibiotic-only, phage-only) and blinded

CFU enumeration where feasible. **Authentication of key resources:** bacterial isolates are speciated and antibiotic-phenotyped at acquisition and periodically re-verified; phage stocks are titer-controlled, sequence/identity-checked, and endotoxin-monitored; the wound-biofilm model is run with validated positive/negative controls. Reagent provenance and lot tracking are maintained.

Human Specimens / Sex as a Biological Variable

This award uses **human-derived bacterial specimens/isolates** (archived or prospectively consented wound-bed cultures), not human-subjects intervention; activities involving identifiable specimens will proceed under IRB review/exemption as applicable, with consent where required. There is no treatment, randomization, or clinical endpoint under this R21, so it is **not a clinical trial** as defined by NIH. Donor sex will be recorded and isolate panels balanced across donor sex where feasible; because bacterial pathogens have no sex, SABV applies at the level of specimen source, and any sex-associated differences in community composition will be reported descriptively.

Vertebrate Animals

No vertebrate animal work is proposed or performed under this R21; Aim 2 relies on in vitro and a single established (non-animal) preclinical wound-biofilm model. Should a future in vivo wound-biofilm study become warranted by the Aim 2 readouts, it will be designed and conducted under a separate, appropriately powered award with its own IACUC-approved protocol — not within this exploratory project.

Clinical-Translation Readiness (Regulatory Path)

Although this R21 performs no human intervention, a central deliverable is a credible first-in-US clinical path. Investigational phage cocktails are unlicensed in the US; any subsequent prospective clinical study would proceed under a **standard investigational IND** (not an emergency-use mechanism), with full IRB review, informed consent, and DSMB oversight appropriate to an early-phase trial. Within this award we will prepare a target product profile, assemble an **FDA pre-IND meeting package** for a topical phage cocktail, and specify a statistically powered Phase II design (eligibility: adults with refractory VLUs or Stage II–IV pressure injuries colonized by cocktail-covered pathogens; primary endpoint safety/tolerability; secondary endpoints wound bioburden and healing). This sequencing front-loads regulatory de-risking so that a future trial can launch promptly on a solid preclinical foundation.

Team & Environment

- **Contact PI [NAME, INSTITUTION]** — phage biology / wound microbiology; overall direction; leads Aims 1–2.
- **Co-Investigator [NAME, INSTITUTION]** — clinical wound care / nursing science; clinical-translation and Phase II design (NINR-aligned).
- **Co-Investigator [NAME, INSTITUTION]** — clinical microbiology / biofilm assays.
- **Regulatory consultant [NAME]** — FDA pre-IND strategy and magistral/GMP-quality phage-preparation planning.
- **Biostatistician [NAME]** — assay design, effect-size/Go-No-Go criteria, and Phase II power analysis.
- **Environment:** academic medical center with BSL-2 microbiology, a wound-care clinic with a long-term-care referral network (for specimen access and future trial siting), and human-subjects/regulatory infrastructure. Potential collaboration with established phage programs [to confirm].

References

1. Rhoads DD, Wolcott RD, Kuskowski MA, Wolcott BM, Ward LS, Sulakvelidze A. Bacteriophage therapy of venous leg ulcers in humans: results of a phase I safety trial. *Journal of Wound Care*. 2009 Jun;18(6):237-43. https://pubmed.ncbi.nlm.nih.gov/19661847/
2. Markoishvili K, Tsitlanadze G, Katsarava R, Morris JG Jr, Sulakvelidze A. A novel sustained-release matrix based on biodegradable poly(ester amide)s and impregnated with bacteriophages and an antibiotic shows promise in management of infected venous stasis ulcers and other poorly healing wounds. *International Journal of Dermatology*. 2002 Jul;41(7):453-8. https://doi.org/10.1046/j.1365-4362.2002.01451.x
3. Karn SL, Bhartiya SK, Pratap A, Saroj SK, Kumar R, Sahu M, Gangwar M, Nath G. A Randomized, Placebo-controlled, Double-blind Clinical Trial of Bacteriophage Cocktails in Chronic Wound Infections. *International Journal of Lower Extremity Wounds*. 2024 (Epub 2024 Jan 17);25(2):427-437. https://doi.org/10.1177/15347346231226342
4. Precisio Biotix Therapeutics, Inc. A Randomized, Double-blind Study to Evaluate the Safety, Tolerability, and Potential Efficacy of BACTELIDE vs. Placebo in Addition to Standard-of-care for S. aureus, P. aeruginosa, and K. pneumoniae Colonized Pressure Injuries. ClinicalTrials.gov Identifier NCT04815798 (Phase 1/2). https://clinicaltrials.gov/study/NCT04815798