Precision Phage Endolysins Against *Porphyromonas gingivalis*: A Biofilm-Targeted, Commensal-Sparing Strategy for Periodontitis

Project Summary / Abstract

Periodontitis is a chronic, dysbiotic biofilm disease driven by the keystone Gram-negative anaerobe *Porphyromonas gingivalis*, which reorganizes the subgingival microbiome and destroys tooth-supporting tissue. The current standard of care—mechanical debridement with adjunctive broad-spectrum antibiotics—loses efficacy in deep periodontal pockets, leaves the subgingival biofilm reservoir intact, indiscriminately depletes health-associated commensals, and promotes antimicrobial resistance. Bacteriophages and phage-derived enzymes offer species-level precision that can spare commensals while degrading biofilm matrix, but no cultured lytic phage that directly infects *P. gingivalis* has yet been reported, and the species harbors prophages and host-defense systems that constrain whole-phage approaches. Building on the 2025 demonstration that phage endolysins computationally identified from the oral virome—delivered as a three-enzyme mixture—inhibit *P. gingivalis* growth in vitro (Xu et al., 2025), this R01 will mature that early signal into a rigorously characterized, biofilm-active, commensal-sparing precision agent. We will (1) mine oral-virome metagenomes for *P. gingivalis*-associated endolysins, recombinantly express and biochemically characterize candidates, and define a lead multi-enzyme cocktail with quantified potency, strain coverage, and stability; (2) test cocktail disruption of *P. gingivalis* mono-species and *P. gingivalis*–*Fusobacterium nucleatum* dual-species biofilms, exploiting evidence that lytic *F. nucleatum* phages disrupt such dual-species biofilms (Kabwe et al., 2025); and (3) validate efficacy and commensal-sparing selectivity in multispecies *ex vivo* subgingival and saliva-derived communities under exposure conditions that anticipate local pocket delivery. By design, this program converts an in silico/in vitro observation into the rigorous preclinical foundation required before any future investigational human use, and it directly serves the NIDCR mission to develop precision, microbiome-conscious therapies for oral disease.

Specific Aims

Periodontitis is a leading cause of adult tooth loss and a source of chronic inflammatory burden, yet

adjunctive antibiotics are blunt, transient in deep pockets, and resistance-promoting. *P. gingivalis* is the keystone pathogen, but no classically cultured lytic phage directly infecting it has been reported, and the species carries a substantial prophage burden—prophages were identified in 24 of 90 sequenced genomes, some active and carrying antibiotic-resistance and virulence genes (Gu et al., 2023)—along with bacterial host-defense systems that, together, constrain whole-phage delivery. Multiple recent reviews converge on the conclusion that isolating a phage directly lytic for *P. gingivalis* remains an unmet first step (Kabwe et al., 2025, narrative review). The most credible near-term path is therefore phage-derived **endolysins**, which act on the cell wall independent of productive phage infection: Xu et al. (2025) computationally identified *P. gingivalis*-infecting phages from oral-virome metagenomes and showed that a mixture of three recombinant endolysins inhibits *P. gingivalis* growth in vitro. We will mature this approach into a biofilm-active, commensal-sparing precision therapeutic candidate.

Central hypothesis: A defined multi-enzyme endolysin cocktail can selectively suppress *P. gingivalis* and degrade its biofilm—including dual-species biofilm with *F. nucleatum*—while sparing health-associated commensals in a community context.

Aim 1. Define a *P. gingivalis*-targeting endolysin cocktail. We will computationally mine oral-virome metagenomic resources for *P. gingivalis*-associated phage endolysins, recombinantly express and purify the most promising candidates, and quantify lytic activity across a diverse panel of *P. gingivalis* strains. We will define per-enzyme and combinatorial potency, strain coverage, and pH/temperature stability to nominate a lead cocktail. *Go/no-go:* a cocktail that produces a defined, reproducible reduction in viable *P. gingivalis* across a majority of panel strains advances to Aim 2.

Aim 2. Determine cocktail efficacy against mono- and dual-species biofilms. We will test whether the lead cocktail penetrates and disrupts *P. gingivalis* mono-species biofilms and *P. gingivalis–F. nucleatum* dual-species biofilms, the latter motivated by evidence that lytic *F. nucleatum* phages disrupt such dual-species biofilms (Kabwe et al., 2025). Endpoints: biofilm biomass, viable-cell killing, and matrix integrity, plus a test of whether combining the cocktail with characterized lytic *F. nucleatum* phages augments scaffold disruption. *Go/no-go:* significant biofilm biomass and viable-count reduction versus vehicle advances the lead to Aim 3.

Aim 3. Validate efficacy and commensal-sparing selectivity in *ex vivo* communities. We will treat multispecies *ex vivo* subgingival biofilms and human saliva-derived communities with the lead cocktail under conditions anticipating local pocket delivery, using community profiling to quantify *P. gingivalis* suppression against off-target effects on health-associated commensals and to define a selectivity window and post-treatment durability.

Impact. Success would yield the first rigorously characterized, biofilm-active, commensal-sparing anti-*P. gingivalis* biologic—a precision alternative to broad-spectrum antibiotics and the preclinical

foundation for future translational development.

Significance

Periodontitis is a chronic dysbiotic biofilm disease in which *P. gingivalis*, a Gram-negative anaerobe of the subgingival "red complex," acts as a keystone pathogen: even at low relative abundance it can reorganize the subgingival microbial community and drive destruction of the periodontal attachment apparatus. Beyond the oral cavity, *P. gingivalis* and its phages have been linked to systemic metabolic disease, including obesity and type 2 diabetes (Xu et al., 2025); broader associations with other systemic conditions have been reported in the literature but are outside the scope of the evidence marshaled here. This oral–systemic relevance makes *P. gingivalis* a high-value target and aligns the work squarely with the NIDCR mission to understand and treat oral disease and its connections to overall health.

The therapeutic gap is concrete and threefold. Standard care—scaling and root planing with adjunctive broad-spectrum antibiotics—(i) loses effectiveness in deep pockets where the biofilm reservoir persists, (ii) depletes the protective commensal flora whose loss perpetuates dysbiosis, and (iii) contributes to antimicrobial resistance. A precision agent that selectively suppresses *P. gingivalis* and degrades biofilm while sparing commensals would address all three failure modes simultaneously.

Phage-based approaches are biologically suited to this problem because phages and their lytic enzymes can be exquisitely species-specific and can penetrate and degrade biofilm matrix. The obstacle is equally concrete: no classically cultured lytic phage directly infecting *P. gingivalis* has been reported. Taxonomically diverse prophages occur in a substantial fraction of sequenced genomes —24 of 90 strains, some active and carrying antibiotic-resistance and virulence genes (Gu et al., 2023)—and bacterial host defenses such as prophage-mediated superinfection exclusion and CRISPR-Cas systems are expected to further limit productive lytic infection. Recent reviews reach the same conclusion: isolating a phage directly lytic for *P. gingivalis* remains an unmet first step (Kabwe et al., 2025, narrative review). This justifies an **endolysin-first** strategy that bypasses the requirement for productive whole-phage infection while the harder isolation problem is pursued in parallel by the field. Critically, the field is presently confined to in silico and in vitro observations; rigorously establishing biofilm activity and commensal-sparing selectivity is the decisive next step, and is the contribution this proposal makes.

Innovation

This proposal is innovative in strategy, not in rhetoric. **First**, it deliberately pivots away from the

stalled whole-phage paradigm toward phage-derived endolysins—the approach with the only concrete 2025 efficacy signal against *P. gingivalis* (Xu et al., 2025). **Second**, it treats endolysins as a defined, multi-enzyme cocktail, engineering coverage and potency across strains rather than relying on a single enzyme, directly extending the reported three-enzyme mixture toward a robust formulation. **Third**, it couples *P. gingivalis*-directed enzymes with consortium-level strategy, exploiting the finding that lytic phages against the bridging organism *F. nucleatum* disrupt *P. gingivalis*-containing dual-species biofilms (Kabwe et al., 2025); simultaneously attacking the keystone pathogen and its biofilm scaffold is conceptually distinct from monotherapy. **Fourth**, it elevates commensal-sparing selectivity to a measured primary endpoint in a community context rather than an assumed property—operationalizing the central advantage of precision antimicrobials. Together these features reframe an early-stage, largely in silico observation into a testable, biofilm-focused therapeutic-development program grounded entirely in current evidence.

Approach

Rigor, reproducibility, and authentication (applies to all Aims). Key biological resources—*P. gingivalis* and *F. nucleatum* strains and recombinant endolysin constructs—will be authenticated by sequence verification and, for strains, by species-confirmatory genotyping; passage number will be tracked. All quantitative assays will use predefined endpoints, vehicle and heat-inactivated-enzyme controls, biological and technical replicates sufficient for prespecified power, and blinded image/enumeration analysis where feasible. For human-derived specimens (Aim 3), donor sex will be recorded and analyzed as a biological variable, and donors of both sexes will be included. Predefined go/no-go milestones (stated per Aim) govern progression.

Aim 1 — Define a *P. gingivalis*-targeting endolysin cocktail

Rationale. Xu et al. (2025) computationally identified *P. gingivalis*-infecting phages from oral-virome metagenomes and demonstrated that a three-enzyme recombinant endolysin mixture inhibits *P. gingivalis* growth in vitro. Because whole-phage delivery is constrained by the prophage burden (Gu et al., 2023) and bacterial host defenses, a defined endolysin cocktail is the most credible lead.

Experimental design. We will mine publicly available oral-virome metagenomic catalogs for candidate *P. gingivalis*-associated endolysins, prioritizing sequences with predicted cell-wall-lytic catalytic and binding domains. Candidates will be recombinantly expressed (testing solubility tags and domain truncations as needed) and purified. Purified enzymes will be screened individually and in combination against a diverse panel of *P. gingivalis* strains using turbidity-reduction and viability (colony-forming) assays under anaerobic conditions. We will define per-enzyme and combinatorial potency, strain coverage, and biochemical stability across the pH and temperature range relevant to the subgingival environment, to nominate a lead multi-enzyme cocktail.

Expected outcomes. A ranked set of expressible, active endolysins; a defined lead cocktail with quantified potency and strain coverage; and stability data supporting downstream formulation.

Go/no-go milestone. A cocktail producing a reproducible, statistically significant reduction in viable *P. gingivalis* across a majority of panel strains advances to Aim 2.

Potential pitfalls & alternatives. Some candidates may not express solubly or may show narrow strain coverage. We will pursue multiple candidates in parallel, test solubility tags and truncations, and—if coverage is limiting—expand cocktail membership or incorporate predicted depolymerase/matrix-active domains. Because the underlying evidence is in vitro/in silico, individual-enzyme attrition is anticipated and treated as expected, not as program risk.

Aim 2 — Determine cocktail efficacy against mono- and dual-species biofilms

Rationale. The clinical reservoir is biofilm, which antibiotics penetrate poorly. Phage-derived enzymes can degrade matrix, and lytic *F. nucleatum* phages disrupt *P. gingivalis*-containing dual-species biofilms (Kabwe et al., 2025), implicating consortium-level strategy.

Experimental design. We will grow *P. gingivalis* mono-species biofilms and *P. gingivalis–F. nucleatum* dual-species biofilms under anaerobic conditions and treat with the Aim 1 lead cocktail across concentrations and exposure times. Endpoints: biofilm biomass (e.g., stain-based quantification), viable-cell counts within biofilm, and matrix integrity assessed by quantitative and imaging assays. We will test combination regimens pairing the endolysin cocktail with characterized lytic *F. nucleatum* phages, comparing simultaneous versus sequential exposure, to probe additive disruption of the dual-species scaffold.

Expected outcomes. Quantified biofilm biomass reduction and *P. gingivalis* killing within biofilm; evidence on whether targeting the *F. nucleatum* scaffold augments cocktail activity.

Go/no-go milestone. Significant biomass and viable-count reduction versus vehicle in mono-species biofilm advances the lead to Aim 3; dual-species and combination results inform, but do not gate, progression.

Potential pitfalls & alternatives. Biofilm may blunt enzyme access relative to planktonic cells. We will optimize dosing and exposure, evaluate sequential versus simultaneous combinations, and incorporate matrix-degrading activities if penetration limits efficacy. Temperate *F. nucleatum* phages may be unsuitable for live combination use; in that case we will rely on lytic isolates and retain the endolysin cocktail as the primary agent.

Aim 3 — Validate efficacy and commensal-sparing selectivity in *ex vivo* communities

Rationale. Precision—suppressing *P. gingivalis* while sparing commensals—is the core therapeutic rationale and must be demonstrated in a community context before any investigational use is contemplated.

Experimental design. We will deploy multispecies *ex vivo* subgingival biofilms and human saliva-derived communities from multiple donors, treating with the lead cocktail under conditions anticipating local pocket delivery (e.g., gel- or rinse-like exposure). Using community profiling (amplicon/metagenomic sequencing with quantitative controls), we will measure *P. gingivalis* suppression against off-target effects on health-associated commensals, defining a selectivity window, and will assess durability of suppression after treatment cessation.

Expected outcomes. Demonstration of selective *P. gingivalis* suppression with limited commensal disruption in a community model, plus a preliminary delivery-relevant exposure profile to guide future translational work.

Potential pitfalls & alternatives. *Ex vivo* communities are variable; we will use multiple donors, record and analyze donor sex as a biological variable, and replicate conditions. If selectivity is incomplete, we will refine cocktail composition to maximize the on-target/off-target ratio. This aim is explicitly preclinical; no human administration is proposed under this award.

Timeline

[ILLUSTRATIVE] **Year 1** — Aim 1 endolysin mining, expression, and characterization; lead cocktail nomination. [ILLUSTRATIVE] **Years 2–3** — Aim 2 mono- and dual-species biofilm efficacy and combination studies. [ILLUSTRATIVE] **Years 3–4** — Aim 3 *ex vivo* community efficacy and selectivity. [ILLUSTRATIVE] **Year 5** — Integration, formulation-relevant exposure profiling, and assembly of the preclinical package to inform any future investigational pathway. Aims overlap to de-risk attrition; go/no-go milestones at the end of Years 1 and 3 gate resource commitment.

Budget Justification (modular R01-style sketch)

[ILLUSTRATIVE] This proposal requests modular direct costs of [ILLUSTRATIVE] $250,000/year for [ILLUSTRATIVE] 5 years. **Personnel:** [ILLUSTRATIVE] PI (2.4 calendar months) for scientific direction; [ILLUSTRATIVE] Co-Investigator with oral-microbiology/biofilm expertise (1.2 months); [ILLUSTRATIVE] one postdoctoral scientist and [ILLUSTRATIVE] one technician (12 months

each) for protein expression, anaerobic culture, and biofilm assays; [ILLUSTRATIVE] partial bioinformatics support for virome mining and community profiling. **Supplies:** anaerobic culture media and *P. gingivalis*/*F. nucleatum* strain panels, recombinant-protein expression/purification reagents, biofilm and imaging consumables, and community-profiling sequencing. **Other:** [ILLUSTRATIVE] sequencing-core fees and publication costs. No vertebrate-animal or human-subjects intervention costs are requested (see below). Final figures will conform to NIH modular budgeting.

Vertebrate Animals

Not applicable. The proposed work is entirely in silico, in vitro, and *ex vivo*; no vertebrate animals are used.

Human Subjects / Clinical Trial

No clinical trial and no administration of investigational product to human subjects are proposed under this award; the program is preclinical, matching the current state of the field (no registered trials or human case reports of phage or phage-enzyme therapy for *P. gingivalis* periodontitis exist as of 2026). Aim 3 uses human-derived saliva/biofilm specimens; any collection or use of such specimens will proceed under Institutional Review Board (IRB) oversight with appropriate informed consent, or will use de-identified samples, and is expected to qualify for exemption where applicable. Donor sex will be recorded and analyzed as a biological variable. For the eventual translational path we note that investigational phage or phage-enzyme therapeutics in the US are typically administered under FDA oversight, including the expanded-access investigational new drug (eIND) route; pursuing such authorization is explicitly outside the scope of this award and would follow only after the preclinical package generated here.

Investigators & Environment

This project requires assembled expertise spanning phage/endolysin biology, anaerobic oral microbiology, biofilm science, and microbial bioinformatics; the assembled team and environment are the principal feasibility drivers for this preclinical program. Template roles to fill with real names/institutions: **Principal Investigator** [NAME, INSTITUTION] — phage-derived enzyme therapeutics; **Co-Investigator** [NAME] — *P. gingivalis* and subgingival biofilm biology; **Co-Investigator** [NAME] — oral-virome metagenomics and endolysin discovery; **Collaborator** [NAME] — *F. nucleatum* phage isolation and dual-species biofilm models; **Bioinformatics support** [NAME/FACILITY]; **Recombinant-protein and sequencing cores** [FACILITY]. The host

institution will provide BSL-appropriate anaerobic microbiology, protein expression and purification, quantitative imaging, and high-throughput sequencing infrastructure. The proposal is grounded in independently published work establishing each pillar: oral-virome endolysin discovery and the three-enzyme anti-*P. gingivalis* mixture (Xu et al., 2025); *F. nucleatum* phage isolation and dual-species biofilm disruption (Kabwe et al., 2025); *P. gingivalis* prophage genomics (Gu et al., 2023); and the field-level rationale and unmet need (Kabwe et al., 2025, narrative review).

References

1. Xu T, Jiao X, Liu G, et al. Oral virome metagenomic catalog links *Porphyromonas gingivalis* phages to obesity and type 2 diabetes. *Cell Reports Medicine*. 2025;6(9):102325. https://doi.org/10.1016/j.xcrm.2025.102325
2. Kabwe M, Tucci J, Dashper S, Binte Mohamed Yakob Adil SS, Petrovski S. Characterisation of novel bacteriophages and their efficacy in disrupting pathogenic dual-species biofilms. *Journal of Oral Microbiology*. 2025;17(1):2584952. https://doi.org/10.1080/20002297.2025.2584952
3. Gu BL, She Y, Pei GK, et al. Systematic analysis of prophages carried by *Porphyromonas gingivalis*. *Infection, Genetics and Evolution.* 2023;113:105489. https://doi.org/10.1016/j.meegid.2023.105489
4. Kabwe M, Tucci J, Darby I, Dashper S. Oral bacteriophages and their potential as adjunctive treatments for periodontitis: a narrative review. *Journal of Oral Microbiology*. 2025;17(1):2469890. https://doi.org/10.1080/20002297.2025.2469890