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LETTER

SPECIAL ISSUE: Oral Regional Immune Mechanisms and Systemic Homeostasis

Engineering bacterial nanosyringes for regional immune recalibration at the oral barrier

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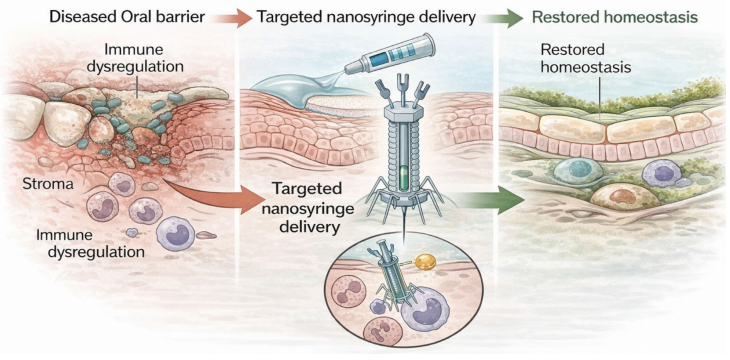
Cite This: *Oral Sci Homeost Med*, **2026**, 2, 9610055 Read Online

ABSTRACT: This article outlines a conceptual and translational framework for adapting engineered bacterial nanosyringes to regional immune recalibration at the oral barrier. The oral cavity is a spatially organized barrier tissue in which epithelial, stromal and immune compartments continuously negotiate microbial exposure, mechanical injury and wound repair. Many oral inflammatory disorders therefore reflect not only microbial imbalance, but also failure of regional immune calibration. Here we propose engineered bacterial nanosyringes, particularly Photorhabdus virulence cassette (PVC)-based extracellular contractile injection systems, as a conceptual framework for cell-targeted immune recalibration at the oral barrier. Rather than presenting these systems as ready-to-use oral therapeutics, we define oral-barrier-specific design requirements, including local retention, cargo compatibility, repeated-dose immunogenicity testing, preservation of host defence and spatially resolved efficacy readouts. We further discuss illustrative cargo modules for transient inflammatory restraint or epithelial repair, while emphasizing that durable recalibration would need to arise from interruption of self-amplifying pathological circuits rather than persistent cargo activity. This framework provides a testable roadmap for adapting bacterial nanosyringe delivery to oral inflammatory disease.



KEYWORDS: oral barrier, regional immune recalibration, bacterial nanosyringes, Photorhabdus virulence cassette, mucosal immunogenicity, targeted delivery

1 Introduction

The oral cavity is better viewed as a sentinel barrier than as an isolated site of disease. It is exposed continuously to microbes, food antigens, frictional injury and tissue turnover, yet in health it maintains a stable, functional equilibrium. Recent work has made clear that this balance is not diffuse, but regionally organized. Integrated spatial and single-cell analyses of human gingiva have revealed marked immune zonation, with location-specific epithelial and stromal programs shaping inflammatory cell organization across the oral mucosal barrier[1,2]. In parallel, contemporary reviews of gingival immunity emphasize that the tissue surrounding the tooth is immunologically unusual: it is chronically challenged by mastication, microbial biofilms and leukocyte trafficking, and disease develops when these local signals are no longer properly contained[3]. In this sense, many oral inflammatory disorders can be understood as disorders of regional immune calibration rather than simple problems of microbial load.

This point matters beyond the mouth. Periodontitis has long been linked to systemic inflammatory disease, but more recent microbiome studies have begun to show how oral inflammation may reshape distal niches as well. In a longitudinal clinical study, patients with generalized stage III/IV periodontitis showed altered oral and gut microbial profiles, and periodontal therapy was associated with significant shifts in gut microbiota toward patterns seen in healthy controls[4]. These observations do not imply a simple one-way causal chain. Rather, they highlight a practical translational premise: if oral inflammation can influence distal microbial or inflammatory states, then recalibrating immune order at the primary barrier site may be more valuable than simply suppressing local bacterial burden. This creates an unmet need for locally confined, cell-selective technologies capable of recalibrating oral barrier immunity rather than broadly sterilizing or suppressing the tissue. Here, the oral-to-systemic inflammatory axis is used primarily to justify why local immune restoration at the oral barrier may have translational relevance beyond local symptom control. It

Received: March 30, 2026; **Revised:** May 15, 2026
Accepted: May 18, 2026

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is not intended as a claim that nanosyringe-based oral intervention will necessarily correct systemic disease. Rather, systemic inflammatory markers, distal microbial shifts and circulating immune activation should be considered exploratory secondary readouts in future proof-of-concept studies.

Current local therapies remain essential, particularly mechanical debridement and locally retained antimicrobial delivery. However, most available periodontal drug-delivery systems have been optimized primarily for antimicrobial exposure, pocket retention and sustained release, rather than for cell-selective intracellular immune modulation[5]. For example, FDA-approved local delivery systems such as Arestin® (minocycline microspheres), PerioChip® (chlorhexidine gluconate), Atridox® (doxycycline gel) or the discontinued Actisite® (tetracycline fiber) rely primarily on sustained antimicrobial or antiseptic release within the periodontal pocket. These modalities can reduce bacterial burden and improve local drug exposure, but they were not designed to deliver defined immunomodulatory cargos into selected epithelial, myeloid or stromal compartments. That gap is especially relevant in oral disease, where barrier failure is shaped by defined cellular compartments, epithelial cells, myeloid cells, stromal organizers and resident lymphocytes, operating in a tightly regional tissue architecture[1,3].

2 Engineered bacterial nanosyringes as an oral-barrier-adapted framework

We propose that oral-targeted engineered bacterial nanosyringes could offer a testable conceptual framework to address this problem. In particular, extracellular contractile injection systems such as the *Photorhabdus* virulence cassette (PVC) provide a plausible foundation for a new local immunoengineering platform. Native PVCs were shown to function as self-contained nanosyringes capable of delivering protein effectors directly into target eukaryotic cells[6]. More recently, engineered PVCs were repurposed as programmable delivery devices for mammalian cells, with high delivery efficiencies reported in optimized cultured-cell or selected experimental settings[7]. These values should be interpreted as evidence of technical programmability rather than as expected delivery performance in saliva-rich, biofilm-associated oral tissues. A subsequent generation of the system further expanded its utility by enabling delivery of diverse biomolecules, including proteins, folded ribonucleoproteins and single-stranded DNA, together with broader retargeting capability *in vitro* and *in vivo*[8]. However, the *in vivo* evidence for oral mucosal application remains preliminary, and the immunogenicity of PVC nanosyringes in the oral mucosa, particularly after repeated local dosing, has not yet been defined. Because the oral barrier is chronically exposed to commensal and pathogenic antigens, future studies will need to assess local inflammatory activation, anti-PVC antibody responses, neutralization of delivery efficiency and off-target tissue reactivity before claims of durable *in vivo* retargeting can be extended to this setting. Importantly, the contribution of this article is not to claim PVC nanosyringes as a de novo technology, but to define how this emerging delivery principle might be redesigned for the oral barrier. The oral cavity differs from previously tested delivery settings in several clinically relevant ways: persistent microbial antigen exposure, salivary clearance, biofilm-associated proteolysis, repeated mechanical injury and highly regionalized immune architecture around the gingiva, sulcus and junctional epithelium. These features require a distinct design logic involving local retention, transient cargo activity, repeated-dose immunogenicity testing and spatially resolved efficacy endpoints. Thus, the central argument is not that PVC nanosyringes are ready-made oral therapeutics, but that the oral barrier provides an accessible yet immunologically demanding setting in which this delivery principle can be evaluated under stringent, site-specific design rules. Taken together, these advances provide a conceptual basis for shifting oral intervention from broad, non-specific treatment toward precise, cell-targeted modulation at the barrier interface (Fig. 1).

3 Translational design requirements

What makes this platform attractive for oral application is not

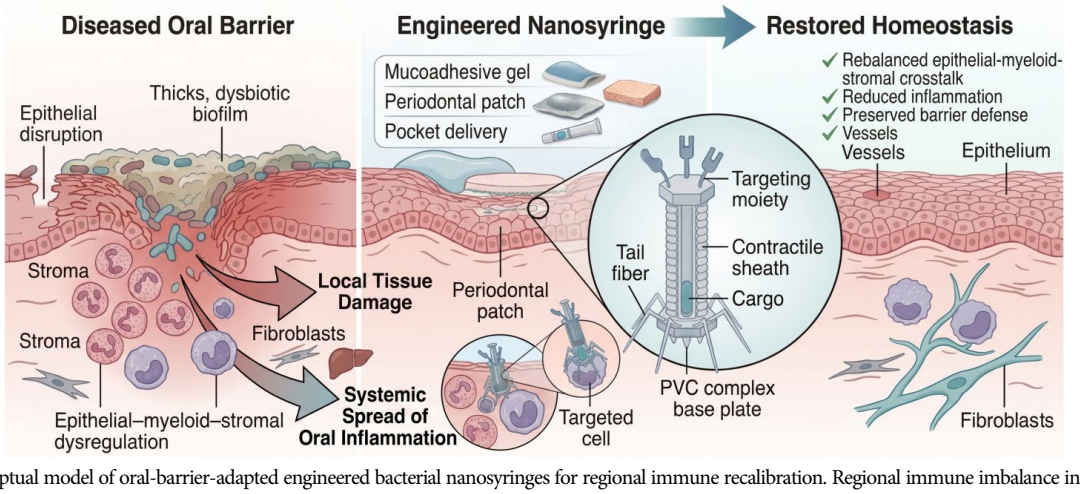

Figure 1 Conceptual model of oral-barrier-adapted engineered bacterial nanosyringes for regional immune recalibration. Regional immune imbalance in the oral barrier is characterized by epithelial disruption, persistent myeloid inflammation, stromal disorganization and dysbiotic biofilm-associated injury. Oral-targeted engineered bacterial nanosyringes may be formulated for local administration, for example as a mucoadhesive gel, film or periodontal-pocket depot, to enable active intracellular delivery of selected cargos to defined cell populations within the oral mucosa. Rather than imposing broad immunosuppression, this strategy is proposed to recalibrate local epithelial–immune–stromal interactions, reduce destructive inflammation and restore barrier integrity while preserving protective host defence. The model should therefore be viewed as a testable engineering framework rather than as evidence that immune homeostasis has already been restored *in vivo*.

novelty alone, but anatomical fit. The oral cavity is accessible, spatially defined and amenable to repeated local administration. For near-term translation, the most realistic format would be a purified, non-replicating PVC formulation incorporated into a mucoadhesive gel, film, patch or periodontal-pocket depot. By contrast, a live microbial chassis engineered for local PVC production would represent a fundamentally different and more complex regulatory pathway. Such a live biotherapeutic format is not the immediate focus of this proposal and would require stringent biocontainment, including auxotrophy, inducible kill switches, environmental dependency and validated clearance mechanisms. These two formats would also raise different regulatory questions. A purified, non-replicating PVC formulation would primarily require characterization of manufacturing consistency, particle purity, local biodistribution, mucosal toxicity, immunogenicity and clearance. A live biotherapeutic PVC-producing chassis would require additional evidence of genetic stability, replication control, environmental containment, shedding behavior, horizontal gene transfer risk and reliable elimination after treatment. For this reason, the non-replicating formulation should be regarded as the more practical first-generation format. Unlike conventional carriers that rely largely on passive uptake, bacterial nanosyringes offer an active cytosolic delivery mechanism. This feature may be useful when the goal is to modulate intracellular programs in selected cell populations rather than simply increase extracellular drug concentration[7,8]. This mode of delivery may also help avoid endosomal sequestration and thereby improve the functional availability of selected cargos in the cytosol[9].

For oral use, immunogenicity should be treated as a design variable rather than a post hoc safety concern. At least three levels of evaluation will be required. First, acute mucosal activation should be measured after single-dose exposure, including epithelial alarmins, neutrophil recruitment, complement activation and local cytokine induction. Second, repeated dosing should be tested for anti-PVC IgA and IgG responses, tissue-resident memory activation, neutralization of delivery efficiency and off-target tissue reactivity. Third, formulation strategies should be compared to minimize unwanted immune activation, including use of purified non-replicating particles, dose minimization, mucoadhesive retention to restrict tissue diffusion and, where feasible, surface engineering to reduce immunodominant bacterial motifs. In this framework, immunogenicity is not simply a limitation of the platform, but a primary criterion for selecting the safest oral formulation and dosing schedule. The oral context makes this balance especially important. Topical delivery may reduce systemic exposure, but repeated application to an antigen-rich mucosal surface may also promote secretory IgA responses, local antigen sampling and neutralization of delivery. Therefore, the goal should not be maximal tissue persistence, but dose-sparing, locally confined and sufficiently brief exposure that allows intracellular delivery while limiting prolonged immune recognition. Mucoadhesive or depot formulations should be optimized for this exposure window rather than simply for maximal retention time.

The therapeutic objective should not be framed as broad immunosuppression. Rather, the aim would be to reset pathological immune microenvironments at the oral barrier. The most relevant initial targets are likely to be oral epithelial cells, infiltrating or tissue-associated myeloid cells, and stromal compartments that regulate leukocyte positioning, inflammatory persistence and tissue repair[1,2]. In periodontal disease, for example, this could mean interrupting destructive inflammatory circuits while preserving host defence and wound-healing capacity. For early-stage oral studies, acceptable anti-PVC immune responses should be defined by functional consequences rather than antibody detection alone. As pragmatic working benchmarks, repeated local dosing should not cause progressive mucosal pathology, sustained cytokine elevation above disease-control levels, systemic inflammatory activation or loss of epithelial barrier integrity. In addition, anti-PVC IgA or IgG induction should be considered acceptable only if it does not produce substantial delivery neutralization, for example a greater than 20–30% reduction in reporter delivery after repeated dosing, or off-target inflammatory reactivity in oral or distal tissues. These thresholds should be refined empirically for each formulation, dose and disease model.

What cargo could achieve this selectivity? At the cargo level, selectivity should come from three layers: cell-selective retargeting, local delivery and transient pathway modulation. Illustrative first-generation cargos could include three functional modules. First, a short-lived IκBα-based inhibitory module or dominant-negative NF-κB regulatory peptide could be used to transiently restrain excessive inflammatory amplification in recruited myeloid cells. Second, a peptide or protein module that disrupts NLRP3–ASC inflammasome assembly could be explored to reduce IL-1β-dominant tissue damage without eliminating upstream microbial sensing. Third, an epithelial repair module, such as a transient junction-stabilizing or wound-healing-supportive peptide, could be directed toward gingival or junctional epithelial cells to reinforce barrier integrity without imposing constitutive proliferative signaling. These examples are not intended to define final therapeutics, but to illustrate how cargo design should match oral disease biology: inflammatory restraint in myeloid cells, barrier reinforcement in epithelial cells and spatial immune reorganization in stromal compartments. The guiding principle is to use rapidly degraded, non-integrating cargos that reduce pathological amplification loops while leaving antimicrobial sensing and wound repair intact. In this regard, oral-targeted nanosyringes sit at an interesting interface between advanced biomaterials and living microbial therapeutics: they combine the local access prized in periodontal drug delivery with a degree of intracellular precision that most current local systems do not readily achieve[5]. This creates an apparent tension: how can transient cargo activity support durable immune recalibration? The intended mechanism is not persistent pharmacological suppression. Rather, transient cargos would be used to interrupt self-amplifying pathological loops, such as IL-1β-dominant myeloid activation, epithelial barrier disruption or stromal chemokine amplification. If these loops are interrupted at the appropriate disease stage, endogenous epithelial repair, microbial containment and stromal reorganization may help sustain a more homeostatic state after the cargo has been degraded. Thus, durability would need to emerge from restoration of local tissue feedback circuits, not from long-term persistence of the delivered cargo. Cargo feasibility must also be considered in relation to PVC packaging and delivery constraints. The most realistic first-generation cargos would likely be compact peptides, small folded protein domains, single-chain inhibitory modules or short-lived protein effectors whose loading, ejection, folding and intracellular activity can be experimentally verified. Larger multi-component signaling systems, unstable proteins or cargos requiring sustained expression would be less suitable for near-term oral application. For each candidate cargo, minimal validation should include packaging

efficiency, particle integrity, delivery into the intended cell type and preservation of biological activity after cytosolic release.

Although periodontitis provides the clearest initial indication because of its spatially accessible inflammatory pocket and well-defined epithelial–myeloid pathology, the same design logic may be adaptable to other oral barrier disorders, including gingivitis, recurrent oral ulceration and selected caries-associated inflammatory states. Gingivitis represents an earlier and potentially reversible epithelial–myeloid inflammatory state; recurrent oral ulceration involves barrier injury, excessive inflammation and defective mucosal repair; and selected caries-associated inflammatory states may involve local dentin-pulp or gingival inflammatory niches rather than enamel demineralization alone. These indications should not be treated as interchangeable. Each would require distinct target cells, cargos, formulations and efficacy benchmarks. In the longer term, oral nanosyringe design could also be individualized according to spatial inflammatory patterns, microbial ecology and barrier-damage signatures, rather than disease labels alone.

This concept also imposes clear translational demands. Oral targeting cannot be generic; the gingiva, junctional epithelium, sulcus and non-keratinized mucosa differ in structure, exposure and immune organization[1-3]. Saliva flow, proteases and biofilm-rich surfaces will challenge particle stability and residence time. If bacterial chassis are considered in future designs, hard safety features, including nonreplicative formats, external control elements and strong containment logic, will be essential. These translational considerations are summarized in Table 1, which has been framed as an oral-barrier-specific design matrix linking local biological constraints to engineering implications and measurable proof-of-concept readouts. Just as importantly, preclinical success should not be judged only by changes in microbial counts or gross inflammatory scores. A more meaningful benchmark is whether treatment restores local immune architecture, epithelial integrity and inflammatory restraint without compromising protective host defence or aggravating oral-to-systemic inflammatory crosstalk[10].

4 Conclusion

In summary, oral-targeted engineered bacterial nanosyringes deserve consideration not as a ready-to-use therapy, but as a testable framework for cell-targeted immune recalibration at the oral barrier. The value of this concept is anchored in a clear translational gap. Oral inflammatory disease is spatial, barrier-based and immune-driven, yet treatment remains dominated by relatively blunt local interventions. An initial proof-of-concept study could proceed in two stages: first, testing locally delivered, purified PVC nanosyringes in ligature-induced periodontitis to determine residence time, tissue penetration, immunogenicity and cell-selective delivery using a reporter cargo; and second, replacing the reporter with a transient pro-resolving cargo to assess whether epithelial integrity, myeloid activation and spatial immune zonation are restored without impairing bacterial containment. Such a study should also test whether a short-lived cargo can produce effects beyond its immediate activity window by measuring not only early pathway inhibition, but also later epithelial repair, myeloid-state normalization, microbial containment and spatial immune reorganization. The immediate goal should be modest but decisive:

Table 1 Oral-barrier-specific design requirements for engineered bacterial nanosyringes.

Oral barrier constraint	Why it matters	Engineering implication	Relevant model/validated assay	Measurable proof-of-concept readout
Saliva flow and mechanical clearance	Reduces local residence time and may dilute or remove particles before delivery	Mucoadhesive gel, film, patch or periodontal-pocket depot	Rodent gingival or periodontal-pocket retention model; fluorescence imaging, qPCR-based particle tracking or labeled reporter biodistribution assay	Quantified local PVC or reporter signal over defined time points, for example 0-72 h, in gingival sulcus, mucosa or periodontal pocket
Biofilm- and protease-rich environment	May degrade PVC particles, targeting elements or cargos	Protective formulation and ex vivo saliva/biofilm stability testing	Ex vivo saliva exposure, protease challenge, biofilm-conditioned medium or multispecies oral biofilm model	Percentage of intact PVC particles and retained cargo activity after exposure to saliva, proteases or biofilm-conditioned medium
Regional immune zonation	Gingiva, sulcus, junctional epithelium and non-keratinized mucosa differ immunologically	Tail-fibre retargeting and formulation should be matched to local tissue context	Ligature-induced periodontitis or LPS-induced gingival inflammation model; flow cytometry, immunofluorescence or spatial transcriptomic/imaging assay	Percentage of reporter-positive epithelial, myeloid and stromal cells by flow cytometry or spatial imaging
Repeated microbial antigen exposure	May amplify anti-PVC mucosal immunity after repeated dosing	Repeated-dose immunogenicity testing and dose-minimizing local retention	Repeated topical dosing model; ELISA for anti-PVC IgA/IgG, multiplex cytokine assay and reporter-delivery neutralization assay	Anti-PVC IgA/IgG titres, mucosal cytokine levels and loss of reporter-delivery efficiency after repeated dosing
Need to preserve host defence	Excessive suppression may worsen infection or delay wound repair	Short-lived, non-integrating, pathway-tuning cargos rather than durable immunosuppression	Ligature-induced periodontitis, oral wound model or mucosal infection-challenge model; CFU/qPCR microbial burden, permeability assay and histology	Oral bacterial burden, epithelial permeability, wound closure rate and histological inflammation score
Disease heterogeneity	Periodontitis, gingivitis, oral ulceration and caries-associated inflammation differ mechanistically	Indication-specific cargo and target-cell selection	Ligature-induced periodontitis, LPS-induced gingivitis, punch-biopsy/acetic-acid oral ulcer model or dentin–pulp inflammation model	Disease-specific endpoints such as alveolar bone loss, gingival inflammatory score, ulcer closure time, pulp/gingival cytokines or microbial containment
Oral-to-systemic inflammatory crosstalk	Local barrier inflammation may associate with distal inflammatory or microbial changes	Treat systemic effects as exploratory, not primary, endpoints	Local periodontitis or gingival inflammation model combined with plasma cytokine profiling, circulating immune phenotyping and distal microbiome analysis	Exploratory plasma cytokines, circulating myeloid activation markers and distal microbiome shifts after local treatment

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to determine whether locally retained, purified PVC nanosyringes can enter defined oral barrier cells, deliver a transient reporter or immunomodulatory cargo, and do so without provoking neutralizing mucosal immunity or impairing host defence. A delivery system capable of acting locally, entering selected cells and modulating defined intracellular programs could open a different therapeutic direction. The goal would not be simply to suppress inflammation, but to recalibrate, rather than broadly override, the immune order of the oral barrier.

Acknowledgements

This study was partly supported by Shenzhen Medical Research Special Fund Project (D2403006), National Natural Science Foundation of China (82473938, U25A20110); Fundamental Research Funds for the Central Universities (24ykzy003).

Data available statement

N/A

Author contribution

G.W. and A.W. conceived the idea and designed the report. H.L., S.Y., M.X. and R.L. performed collection of reference and summary; G.W. provided administration and supervision. A.W. wrote the manuscript. All authors have read and approved the manuscript.

Ethics approval and consent

N/A

Consent for publication

All authors agree to publish.

Conflicts of interest

The authors declare that they have no conflict of interest.

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