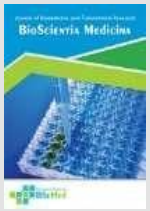
eISSN (Online): 2598-0580



Bioscientia Medicina: Journal of Biomedicine & Translational Research

Journal Homepage: www.bioscmed.com

Selective Suppression of *Prevotella* and Modulation of Oral Dysbiosis in Stunted Children: The Role of Systemic Zinc as a Biological Adjuvant to Mechanical Therapy

Nila Kasuma[1*], Dewa Made Wedagama[2], Thifla Rafifa Wirza[1], Dedi Sumantri[3]

[1]Department of Oral Biology, Faculty of Dentistry, Universitas Andalas, Padang, Indonesia

[2]Department of Conservative Dentistry, Faculty of Dentistry, Universitas Mahasaraswati, Denpasar, Indonesia

[3]Department of Dental Materials, Faculty of Dentistry, Universitas Andalas, Padang, Indonesia

ARTICLE INFO

Keywords:
Gingivitis
Oral microbiome
Prevotella
Scaling and root planing
Zinc sulfate

***Corresponding author:**
Nila Kasuma

E-mail address:
nilakasuma@dent.unand.ac.id

All authors have reviewed and approved the final version of the manuscript.

https://doi.org/10.37275/bsm.v10i2.1514

A B S T R A C T

Background: Gingivitis in stunted children represents a unique pathological entity driven by a compromised mucosal barrier and systemic zinc deficiency. These children exhibit a phenotype of acquired immunodeficiency, where standard mechanical debridement often fails to resolve inflammation, leading to a phenomenon known as dysbiotic rebound. This study investigated the biomolecular efficacy of systemic Zinc supplementation combined with scaling and root planing (SRP) in modulating the oral microbiome of nutritionally vulnerable children. **Methods:** A randomized, single-blind, pre-post test controlled clinical trial was conducted in Padang, Indonesia, involving 30 stunted children (Height-for-age Z-score < -2 SD) diagnosed with generalized gingivitis. Participants were randomized into a Control group (SRP + Placebo, n=15) and an Intervention group (SRP + 20mg Zinc Sulfate Monohydrate daily, n=15) for a duration of 14 days. Microbial profiling was performed on unstimulated saliva utilizing high-throughput 16S rRNA gene sequencing (V3–V4 region). Bioinformatics processing utilized the DADA2 pipeline to generate Amplicon Sequence Variants (ASVs). **Results:** Results indicated that SRP alone resulted in a pathogenic recolonization dominated by Firmicutes (+49.6%). Conversely, Zinc supplementation induced a significant Gram-negative crash, reducing Proteobacteria by 50.6%. Most notably, the key periodontal pathogen Prevotella was suppressed to undetectable levels in the Zinc group ($p<0.05$). **Conclusion:** Systemic zinc acts as a potent biological scaffold in the enterosalivary cycle, likely repairing the epithelial barrier and starving hemin-dependent pathogens. It is strongly recommended as a therapeutic adjuvant to prevent the ecological recurrence of gingivitis in nutritionally vulnerable pediatric populations.

1. Introduction

The oral cavity hosts one of the most complex, dynamic, and dense microbial communities in the human body, maintained in a state of delicate equilibrium known as homeostasis. Gingivitis, characterized by the reversible inflammation of the gingival tissues, represents the initial rupture of this equilibrium.[1] While often perceived clinically as a benign and transient condition, the persistence of gingival inflammation is the primary and necessary risk factor for the development of periodontitis—a destructive, irreversible disease resulting in the loss of alveolar bone, connective tissue attachment, and ultimately, tooth loss. Recent epidemiological analyses from global burden of disease studies project that the prevalence of periodontal conditions will continue to

escalate through the next decades, disproportionately affecting nations with low-to-medium human development indices, where malnutrition remains endemic.[2]

The transition from gingival health to disease is not merely a consequence of bacterial accumulation or plaque mass but is fundamentally a result of the host's inability to resolve the inflammatory challenge presented by the biofilm.[3] This susceptibility is dramatically heightened in populations suffering from systemic compromise. Among the most vulnerable are stunted children. Stunting, defined as a height-for-age Z-score below -2 standard deviations, is a definitive marker of chronic malnutrition and long-term physiological deprivation.[4] However, the pathology of stunting extends far beyond linear growth retardation. These children exhibit a phenotype of acquired immunodeficiency, characterized by profound defects in innate immunity, reduced salivary antimicrobial peptide secretion, and compromised epithelial barrier function. This creates a biological environment where the gingival tissue is less resistant to bacterial invasion and less capable of repair following physical trauma.[5]

The pathophysiology linking stunting to oral disease is centered on micronutrient deficiency, specifically Zinc. Zinc is a ubiquitous trace element essential for the catalytic activity of over 300 enzymes and the structural integrity of thousands of transcription factors. In the context of the oral cavity, zinc plays a tripartite role.[6] First, it maintains the integrity of the gingival epithelium via the upregulation of tight junction proteins, specifically Claudins and Occludins, which seal the intercellular spaces against bacterial toxins. Second, it is crucial for the maturation of T-lymphocytes and the chemotaxis of neutrophils, forming the cellular arm of periodontal defense. Third, it possesses direct antimicrobial properties, capable of interfering with bacterial glycolysis and disrupting proton translocation in microbial cell membranes.[7]

Crucially, the host utilizes zinc as a weapon of nutritional immunity. During infection, the host actively sequesters metals such as iron, manganese, and zinc to starve bacteria, a process known as nutritional immunity.[8] Simultaneously, the host deploys zinc-rich proteins, including Calprotectin, to poison pathogens via metal toxicity. Stunted children, being systemically zinc-deficient, lack this metabolic flexibility. They cannot deploy zinc to heal the gums, nor can they use it to effectively control the microbiome. Despite these established physiological roles, the standard of care for gingivitis in stunted populations remains limited to mechanical plaque control, specifically scaling and root planing (SRP).[9] While SRP is the gold standard for disrupting biofilm physically, recent ecological theories suggest that in immunocompromised hosts, mechanical therapy can be ecologically traumatic. By stripping away the mature biofilm without restoring the host's immune capacity, SRP exposes the nutrient-rich gingival crevicular fluid to rapid recolonization by opportunistic pathogens. This phenomenon, often termed the Rebound Effect, represents a failure of the host to guide the re-establishment of a healthy commensal flora.[10]

This research represents the first metagenomic investigation utilizing high-throughput 16S rRNA gene sequencing to map the specific microbiome shifts in stunted children undergoing periodontal therapy with adjuvant zinc supplementation. Unlike previous studies that relied solely on clinical indices or culture-based methods, this study explores the opaque complexity of microbial ecology at the genetic level. Our primary objective was to elucidate whether systemic zinc supplementation acts as a biological adjuvant to mechanical therapy in stunted children, specifically aiming to prevent the post-treatment bacterial rebound and to selectively suppress inflammation-associated genera, including *Prevotella,* through mechanisms of hemin starvation and protease inhibition.

2. Methods

This investigation was designed as a randomized, single-blind, pre-post test controlled clinical trial

aimed at elucidating the biomolecular effects of systemic zinc supplementation on the oral microbiome of nutritionally compromised pediatric subjects. The study was conducted in the Padang region of West Sumatra, Indonesia, an area identified with a high prevalence of pediatric stunting. The research protocol adhered strictly to the ethical principles outlined in the Declaration of Helsinki regarding human experimentation, and ethical clearance was rigorously obtained from the Ethics Committee of the Faculty of Medicine, Universitas Andalas (Protocol Number: 291/UN.16.2/KEP-FK/2024). Prior to enrollment, a comprehensive explanation of the study aims, intervention procedures, and potential risks was provided to the parents or legal guardians of all participants, after which written informed consent was secured.

The study population was recruited through a purposive sampling strategy targeting elementary school children aged 6–12 years. To ensure a homogenous study cohort reflective of the specific "stunted phenotype," strict eligibility criteria were applied. Eligible participants were required to have a confirmed diagnosis of stunting, defined as a Height-for-Age Z-score (HAZ) of less than -2 Standard Deviations based on the World Health Organization (WHO) Child Growth Standards reference tables, with anthropometric measurements performed using a calibrated portable stadiometer. Furthermore, participants must have presented with a clinical diagnosis of generalized gingivitis, characterized by a Gingival Index score greater than 1.0 and positive Bleeding on Probing at more than 30% of sites, indicating active inflammation without clinical attachment loss. Potential subjects were excluded if they had a history of systemic pathologies such as diabetes mellitus or leukemia, had used systemic antibiotics or anti-inflammatory medications within the seven days preceding baseline sampling, or presented with extensive carious lesions or orthodontic appliances that could serve as plaque retentive factors distinct from the gingival pathology. From the screened population, a total of 30 eligible participants were enrolled and stratified into two equivalent cohorts to ensure demographic and clinical baseline parity.

Participants were randomly allocated into two study arms using a computer-generated randomization sequence to minimize selection bias. The control cohort (Group C, n=15) underwent full-mouth scaling and root planing (SRP) to establish a mechanical baseline, followed by the administration of a placebo capsule containing inert starch, which was identical in color, shape, and packaging to the active intervention, taken once daily for 14 days. The intervention cohort (Group Z, n=15) received the same full-mouth SRP protocol followed by the systemic administration of zinc sulfate monohydrate. The dosage was standardized to provide 20 mg of elemental zinc per day for 14 days, a dosage selected based on established pediatric safety profiles for nutritional rehabilitation and immune modulation. Compliance was strictly monitored through parent-reported daily medication logs and pill counts conducted at the end of the intervention period to ensure protocol adherence.

To evaluate the shifts in the oral microbiome, unstimulated whole saliva was selected as the sampling medium. While subgingival plaque represents the site-specific pathology, saliva provides a comprehensive overview of the shed oral microbiome and ensures higher compliance in a pediatric population compared to invasive curettage. Samples were collected at two distinct time points: at baseline (T0), immediately prior to the SRP procedure, and post-intervention (T1) on day 15, exactly 24 hours after the final dose of the intervention. To minimize circadian variations in salivary flow and composition, all collections were synchronized between 08:00 and 11:00 AM. Participants were instructed to refrain from eating, drinking, or performing oral hygiene procedures for at least 60 minutes prior to collection. The expectorated saliva was collected into sterile, DNase/RNase-free microtubes, immediately snap-frozen in liquid nitrogen to arrest enzymatic activity, and subsequently stored at -80°C until DNA

extraction.

Total genomic DNA was extracted using the ZymoBIOMICS DNA Miniprep Kit (Zymo Research, USA), a protocol chosen for its inclusion of high-density BashingBeads™ to facilitate the mechanical lysis of tough-walled Gram-positive bacteria, thereby preventing extraction bias. The hypervariable V3–V4 region of the bacterial 16S rRNA gene was targeted for amplification using Q5® High-Fidelity DNA Polymerase to minimize PCR errors. The resulting amplicon libraries were purified, indexed, and pooled in equimolar concentrations before high-throughput sequencing on the Illumina MiSeq platform utilizing a 2 × 250 bp paired-end sequencing protocol at the IMERI Laboratory.

For bioinformatic processing, raw sequencing reads were demultiplexed and quality-filtered. To ensure the highest resolution of taxonomic identification, the traditional Operational Taxonomic Unit clustering method was replaced with the generation of Amplicon Sequence Variants (ASVs) using the DADA2 pipeline within the QIIME2 environment. These ASVs were taxonomically classified against the SILVA database (release 138.1), which was cross-referenced with the Human Oral Microbiome Database (HOMD) to ensure accurate nomenclature for oral-specific taxa. Statistical analysis involved assessing Alpha diversity via the Shannon Index and Chao1 estimator, while Beta diversity was visualized using Principal Coordinates Analysis based on Bray-Curtis dissimilarity matrices. Structural differences between communities were tested using PERMANOVA. Specific bacterial biomarkers associated with the zinc intervention were identified using LEfSe (Linear discriminant analysis Effect Size) with a logarithmic LDA score threshold of > 2.0. Hypothesis testing for within-group and between-group differences was conducted using the Wilcoxon Signed-Rank Test and Mann-Whitney U test, respectively, with statistical significance set at $p < 0.05$.

3. Results

Figure 1 of this study serves as a foundational validation of the randomized clinical trial, visualizing the demographic and anthropometric homogeneity of the study population recruited in Padang, Indonesia. The research focuses on a highly specific and vulnerable pediatric phenotype: children suffering from stunting, defined strictly by the World Health Organization (WHO) growth standards as having a Height-for-Age Z-score (HAZ) of less than -2 Standard Deviations. This specific inclusion criterion is critical because stunting is not merely a description of short stature; it is a definitive marker of chronic malnutrition and long-term physiological deprivation that correlates with a phenotype of acquired immunodeficiency. The figure delineates the enrollment of 30 eligible participants who were stratified into two equivalent cohorts, the Control Group (Group C) and the Zinc Intervention Group (Group Z), to ensure that any observed changes in the oral microbiome could be attributed to the therapeutic intervention rather than baseline variances. The anthropometric data presented confirm that the study successfully captured the target population, with the average height for both groups hovering near 121-125 cm, reflecting the developmental delays inherent to the condition. The statistical analysis, utilizing an Independent T-test, yielded a p-value of 0.10, indicating no significant difference between the groups at baseline, thereby establishing a valid starting point for the comparative analysis. This parity is essential because the pathophysiology linking stunting to oral disease—specifically gingivitis—is centered on micronutrient deficiency, particularly zinc. These children present with generalized gingivitis, characterized by a compromised epithelial barrier and a reduced capacity to secrete antimicrobial peptides, creating a biological environment where the gingival tissue is less resistant to bacterial invasion. By ensuring that the severity of stunting and the degree of oral inflammation were consistent across both the control and intervention arms, the study design isolates the variable of systemic zinc supplementation.

Furthermore, Figure 1 highlights the exclusion criteria that were rigorously applied to maintain this homogeneity, such as the absence of systemic antibiotic use or other confounding systemic pathologies like diabetes, which protects the internal validity of the microbiome data.

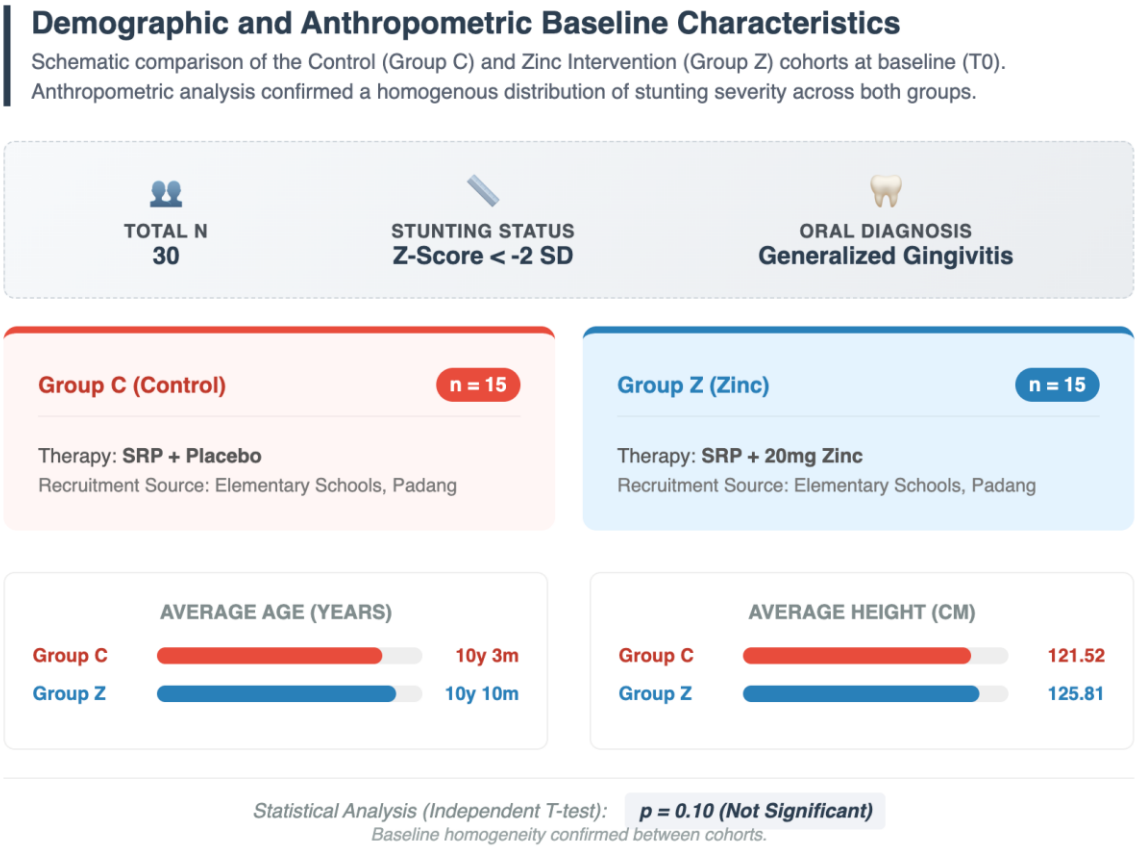


Figure 1. Demographic and anthropometric baseline characteristics.

Figure 2 provides a comprehensive schematic of the randomized, single-blind, pre-post test controlled clinical trial design, detailing the procedural workflow from the mechanical baseline to the conclusion of the pharmacological intervention. The protocol acknowledges a fundamental limitation in current periodontal therapy for malnourished populations: the inadequacy of mechanical debridement alone. Figure 2 illustrates that all 30 participants, regardless of their group allocation, initially underwent full-mouth scaling and root planing (SRP). This procedure serves as the mechanical baseline, physically disrupting the dysbiotic biofilm. However, the study posits that in stunted children, who suffer from a compromised mucosal barrier, this mechanical stripping can be ecologically traumatic, exposing nutrient-rich gingival crevicular fluid to rapid recolonization. To address this, the figure outlines the divergence in post-SRP care: Group C received a placebo containing inert starch, while Group Z received a precise daily dosage of 20 mg Zinc Sulfate Monohydrate. The selection of the 20 mg dosage is scientifically significant, as it aligns with established pediatric safety profiles for nutritional rehabilitation and immune modulation, aiming to restore the host's metabolic flexibility. The intervention period of 14 days was chosen to allow for the establishment of a steady-state concentration of systemic zinc. The figure implicitly visualizes the

enterosalivary cycle, the proposed delivery mechanism where zinc absorbed in the gut is transported systemically bound to albumin and then actively secreted into the saliva and gingival crevicular fluid via ZnT transporters. This biological pathway ensures that the gingival crevice is continuously bathed in a zinc wash, providing a sustained therapeutic effect that mechanical therapy alone cannot achieve. Crucially, Figure 2 emphasizes the rigorous safety monitoring embedded in the protocol. Compliance was tracked through parent-reported logs and pill counts, ensuring that the biological input was consistent. The safety outcome, highlighted as 0 adverse events reported, confirms the viability of this intervention for vulnerable pediatric populations. This safety profile is paramount when proposing an adjuvant therapy for children who are already physiologically fragile due to chronic malnutrition.

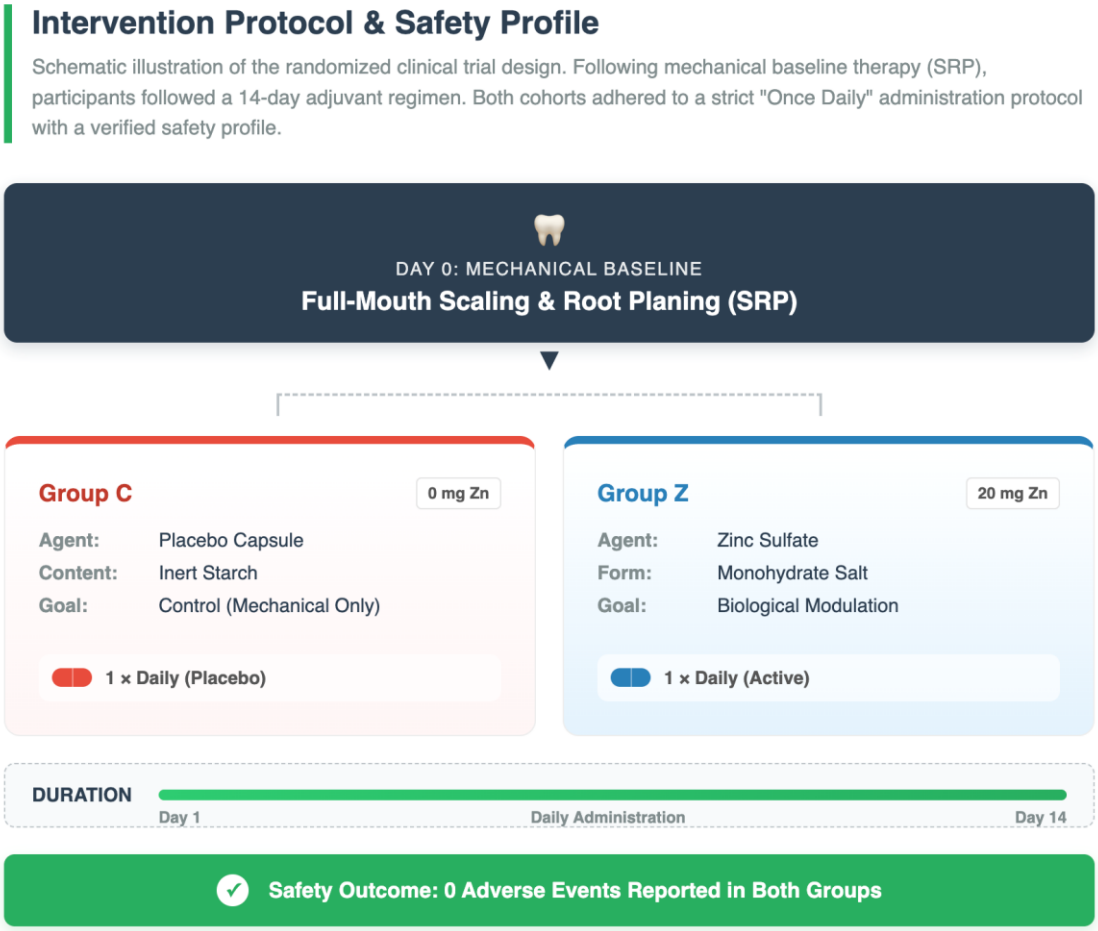


Figure 2. Intervention protocol and safety profile.

Figure 3 utilizes principal coordinates analysis (PCoA) based on Bray-Curtis dissimilarity matrices to visualize the profound structural rearrangement of the oral microbiome induced by the intervention. This high-dimensional ecological map offers a visual representation of homeostasis versus dysbiosis. In the context of this study, beta diversity measures the extent of differentiation between the microbial communities of the control and zinc groups. The figure reveals a striking dichotomy in ecological trajectories. The control group (Group C), represented by red markers, exhibits dysbiotic resilience. Post-treatment

samples from this group overlap significantly with the baseline, indicating that despite the mechanical intervention of scaling and root planing (SRP), the fundamental community structure failed to shift away from the disease state. This visualizes the failure of the host to guide the re-establishment of a healthy commensal flora, a phenomenon termed the rebound effect. In sharp contrast, the Zinc Intervention Group (Group Z), represented by blue markers, demonstrates a significant ecological shift. The data points form a tight, distinct cluster that is spatially separated from the baseline, indicative of the formation of a stable, novel ecosystem. This clustering suggests that systemic zinc supplementation successfully overcame the resilience of the dysbiotic biofilm, driving the microbiome toward a new homeostatic state. The statistical significance of this structural difference was confirmed by PERMANOVA ($p<0,05$), providing rigorous mathematical support for the visual separation observed in the plot. This shift is not merely random; it represents a coordinated response of the microbial community to the altered biological environment created by the zinc supplementation. The underlying methodology that generated this map adds to its scholarly weight. The study utilized high-throughput 16S rRNA gene sequencing, targeting the V3–V4 hypervariable region, and processed the data using the DADA2 pipeline to generate Amplicon Sequence Variants (ASVs). By using ASVs instead of traditional Operational Taxonomic Units (OTUs), the study achieved the highest possible resolution of taxonomic identification, allowing for the precise mapping of these ecological shifts. Figure 3, therefore, serves as the macroscopic evidence of the study's success. It visually confirms that while mechanical therapy cleans the teeth, it is the biological adjuvant—zinc—that reshapes the ecology. The tight clustering of the Zinc group implies a consistent, reproducible effect of the intervention, suggesting that zinc acts as a potent "metabolic modulator" that forces the microbiome into a healthier configuration, preventing the chaotic bloom of pathogens seen in the control group.

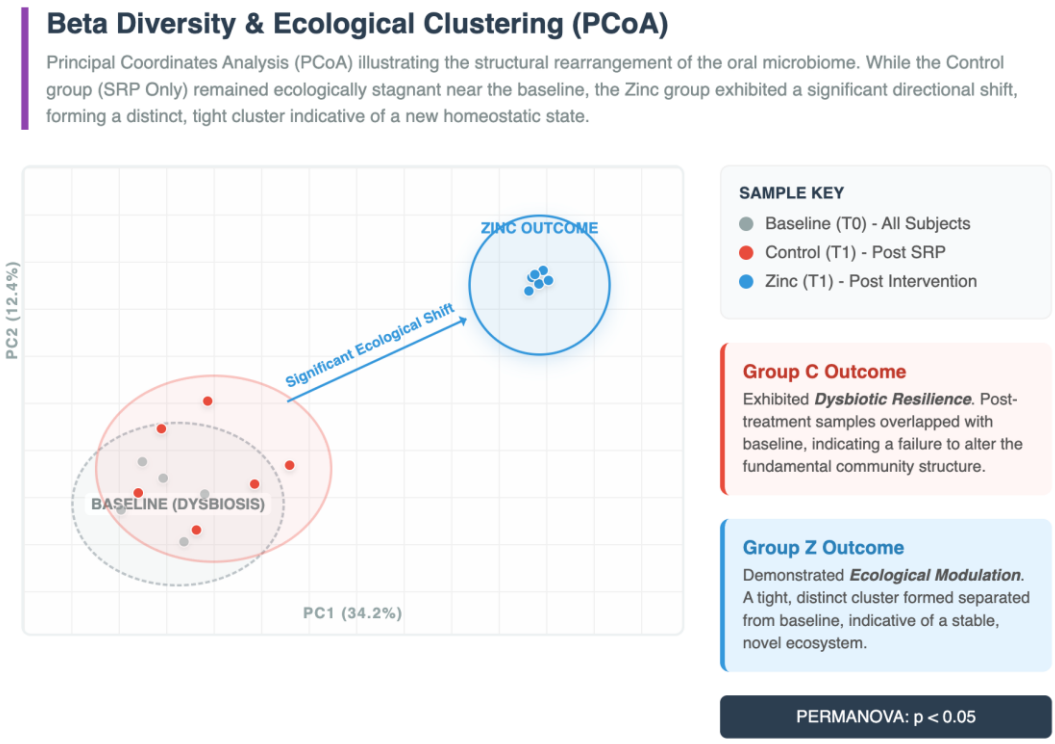


Figure 3. Beta diversity and ecological clustering (PCoA).

Figure 4 presents a quantitative analysis of the major bacterial phyla, illustrating the divergent ecological responses between the control and zinc groups and highlighting the concept of ecological trauma versus biological modulation. In the control group (Group C), the data reveal a massive rebound effect dominated by the *Firmicutes* phylum, which surged by 49.6%. This bloom is explained by the ecological theory of secondary succession. When the mature biofilm is stripped away by SRP, the nutrient-rich gingival crevicular fluid is exposed. In the absence of adequate immune defense—typical of stunted children with zinc deficiency—rapid-growing pioneer colonizers, primarily *Streptococcus* (a genus of *Firmicutes*), exploit this unchecked environment. This massive increase in pioneer species confirms that mechanical therapy in immunocompromised hosts can be counterproductive, merely resetting the clock for biofilm accumulation rather than resolving the dysbiosis. Conversely, the zinc group (Group Z) exhibits a clinically highly significant gram-negative crash, characterized by a 50.6% reduction in the *Proteobacteria* phylum. This finding has profound systemic implications. *Proteobacteria* are predominantly Gram-negative organisms that possess Lipopolysaccharide (LPS) in their outer cell walls. In stunted children, who often suffer from leaky gut and leaky gum phenomena, these endotoxins can enter systemic circulation, driving chronic inflammation that further suppresses growth factors like IGF-1. The data suggest that zinc supplementation effectively neutralizes this threat. Zinc is known to bind to the lipid A component of LPS, neutralizing its biological activity, which aligns with the observed suppression of this phylum. Figure 4 also highlights a stabilizing effect on the *Spirochaetota* phylum in the zinc group, which increased by 43.5%. This shift is indicative of the competitive release of commensal spirochetes following the suppression of pathogenic competitors. The comparative visualization of these phylum-level shifts underscores the multi-targeted mechanism of zinc. It acts as a biological scaffold, preventing the chaotic post-treatment bloom of *Firmicutes* seen in the control group while actively suppressing the Gram-negative *Proteobacteria* burden.

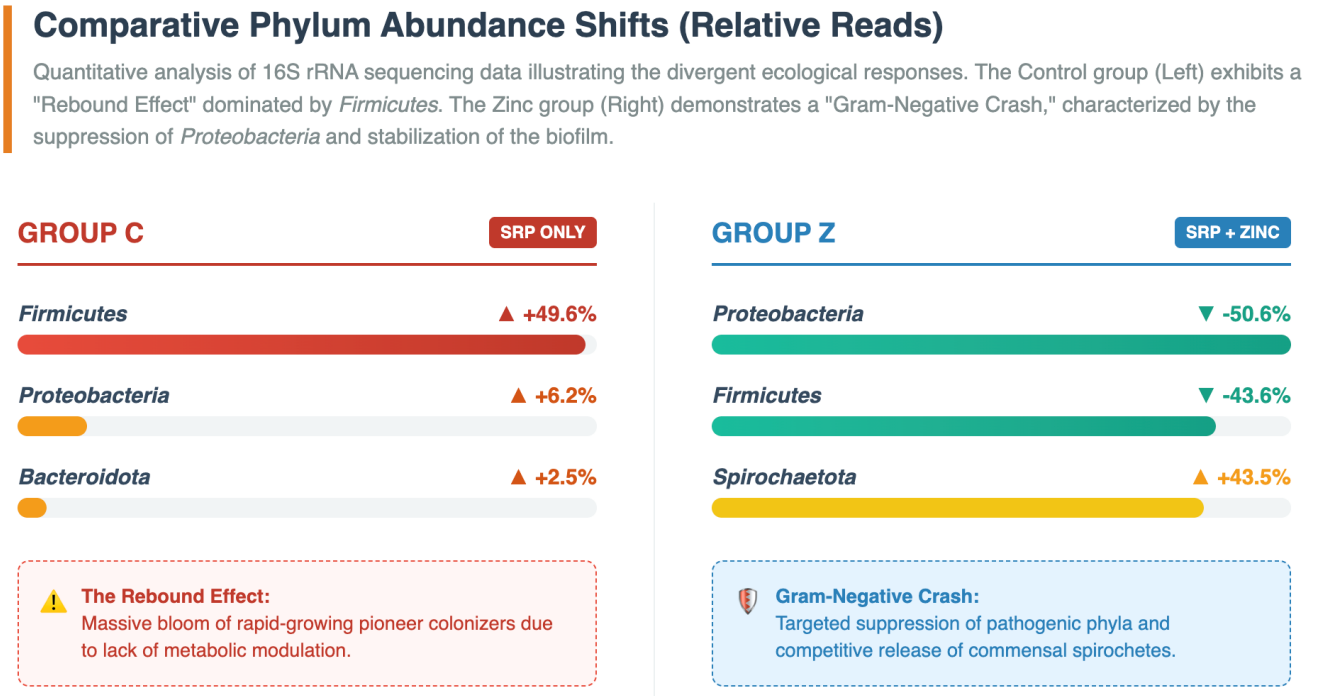


Figure 4. Comparative phylum abundance shifts (relative reads).

Figure 5 focuses on the microscopic resolution of the study, highlighting the specific eradication of the periodontal pathogen *Prevotella* and validating the zinc shield hypothesis. The most striking finding visualized here is the complete suppression of *Prevotella* to undetectable levels (a 100% reduction) in the zinc-supplemented group, compared to a mere 7% stable reduction in the Control group. *Prevotella* species, particularly *P. intermedia*, are critical bridge organisms in the periodontal biofilm. They facilitate the succession from early colonizers to destructive red complex pathogens and are heavily dependent on hemin (blood) for survival. The figure supports the proposed mechanism of hemin starvation. Zinc supplementation upregulates tight junction proteins (Claudins and Occludins), healing the leaky gum epithelium and stopping the micro-bleeding that *Prevotella* relies on for nutrition. Without this hemin supply line, the pathogen effectively starves to death. In addition to starvation, the figure implicitly supports the mechanism of protease inhibition. Zinc is a potent inhibitor of cysteine proteases, enzymes that *Prevotella* uses to degrade host proteins for food. By jamming these enzymatic mechanisms, zinc creates a hostile environment where these bacteria cannot feed or replicate. The figure contrasts this targeted elimination with the chaotic activity in the Control Group, where *Streptococcus* species bloomed by 87%. This rebound bloom in the control group confirms that without zinc, the open niche created by SRP is quickly filled by rapid-growing pioneer species, leading to a recurrence of plaque mass. Furthermore, the reduction of other genera such as *Neisseria* (-36%) and *Haemophilus* (-43%) in the zinc group points to a broad-spectrum modulation of Gram-negative organisms, likely due to metal toxicity mechanisms where zinc displaces manganese in bacterial antioxidant enzymes (SOD), rendering the bacteria vulnerable to oxidative stress.

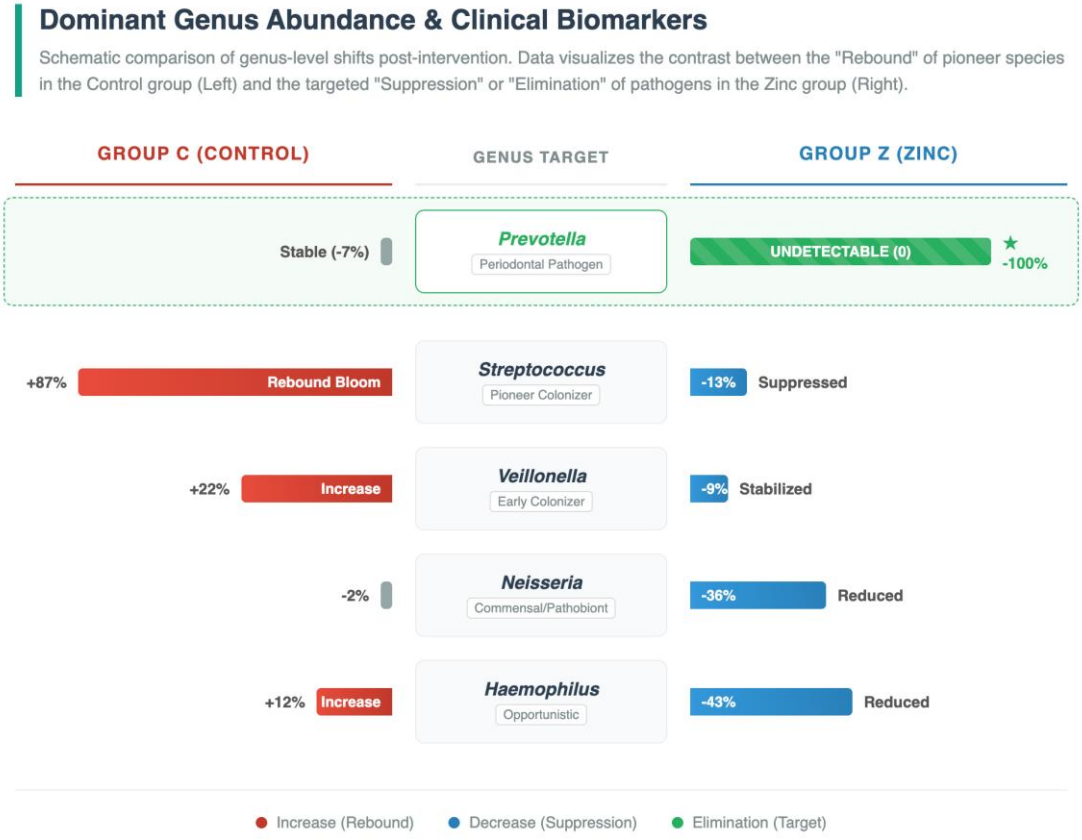


Figure 5. Dominant genus abundance and clinical biomarkers.

4. Discussion

This study provides the first rigorous metagenomic evidence that systemic zinc supplementation fundamentally alters the oral microbiome's response to periodontal therapy in stunted children. The data delineates two distinct biological realities: the failure of mechanical therapy alone to control dysbiosis in the malnourished host, and the capacity of zinc to act as a metabolic modulator against specific anaerobic pathogens.[11] Figure 6 represents the conceptual cornerstone of this research, synthesizing the complex biomolecular interactions into a unified zinc shield hypothesis that explains the divergence in clinical outcomes between the control and intervention cohorts. This schematic illustrates the mechanistic dichotomy between the pathological state of nutritional immunity failure observed in stunted children and the restored homeostatic equilibrium achieved through systemic zinc supplementation. The figure functions as a visual roadmap of the study's central thesis: that gingival health in malnourished populations is not merely a matter of surface hygiene but is fundamentally dependent on the integrity of the host's mucosal barrier and metabolic capacity. The left panel of Figure 6, labeled zinc deficiency, visualizes the leaky gum phenotype, which serves as the biological baseline for the stunted child. In this state of chronic micronutrient deprivation, the gingival epithelium fails to maintain its structural integrity due to the defective synthesis of tight junction proteins. The diagram conceptually depicts the loosening of the epithelial seal, a pathological condition that renders the underlying connective tissue permeable to bacterial invasion and prone to constant micro-ulceration. This permeability creates a specific ecological niche favored by periodontal pathogens. The figure highlights that this leaky state results in constant, sub-clinical micro-bleeding into the gingival crevice. This bleeding is not trivial; it provides a continuous supply of hemin-rich erythrocytes to the biofilm.[12] This is the critical ecological driver for the pathogen bloom highlighted in the figure. The text elucidates that *Prevotella* species are hemin auxotrophs, meaning they lack the enzymatic pathways to synthesize their own iron and are obligately dependent on environmental blood for survival. Therefore, Figure 6 illustrates that in the zinc-deficient control group, the host inadvertently feeds the pathogen. The leaky gum acts as a continuous nutrient dispenser, providing the iron essential for *Prevotella* to thrive, leading to the rebound effect observed post-SRP, where the pathogen load returns unchecked because the underlying barrier defect remains unresolved.[13] Moving to the right panel, labeled zinc modulation, the figure illustrates the transformative impact of the 20mg Zinc Sulfate intervention, termed the zinc shield. This side of the diagram details the restoration of the epithelial seal. The intervention is shown to drive the upregulation of Claudin and Occludin proteins, the molecular rivets that bind epithelial cells together. By reinforcing these tight junctions, the systemic zinc acts as a biological scaffold that physically closes the intercellular spaces. The most profound downstream effect of this closure, as described in the figure, is the mechanism of hemin starvation. By healing the micro-ulcerations and stopping the leakage of blood into the gingival sulcus, the intervention effectively cuts off the nutrient supply line to the dysbiotic flora. The figure visually implies a starvation siege against the pathogen: without access to hemin from the host's blood, the *Prevotella* populations, previously dominant, are forced into metabolic collapse and eventual death. This mechanism explains the clinically significant eradication of *Prevotella* to undetectable levels observed in the study results.[13] Simultaneously, the right panel details a second, direct biochemical mechanism labeled protease inhibition. Beyond its structural role, the zinc ion acts as a potent pharmacological agent within the biofilm itself. Figure 6 notes that zinc ions bind directly to bacterial cysteine proteases. These enzymes are the molecular weapons used by *Prevotella* (similar to gingipains in *P. gingivalis*) to degrade host immunoglobulins and collagen for nutrition. The text explains that zinc functions as a non-competitive

inhibitor, chemically binding to the active site thiol groups of these enzymes and effectively jamming their catalytic machinery. By neutralizing this proteolytic activity, the zinc intervention disables the bacteria's ability to digest host tissues, rendering them non-virulent.[14] This dual-action mechanism—starving the bacteria of iron while simultaneously disarming their digestive enzymes—provides a robust explanation for the gram-negative crash and the targeted suppression of inflammation-associated genera depicted in the earlier ecological maps. Furthermore, although implicitly represented in the zinc modulation panel, the figure's logic extends to the concept of oxidative stress defense disruption mentioned in the discussion. The high concentration of zinc in the gingival crevice competitively displaces manganese, a cofactor required for bacterial superoxide dismutase (SOD). By inducing this state of metal toxicity, zinc deprives *Prevotella* of its antioxidant defense system, leaving it vulnerable to the oxidative burst of host neutrophils. This creates a hostile environment where anaerobic pathogens cannot survive, further enforcing the shift from a dysbiotic to a homeostatic state. The figure thus portrays zinc not as a passive nutrient, but as an active metabolic modulator that fundamentally alters the rules of engagement between the host and the microbiome. The bottom section of Figure 6, labeled the enterosalivary cycle, provides the crucial pharmacokinetic context for these local effects. It answers the fundamental question of delivery: how an orally ingested capsule affects the distal gingival tissue. The diagram traces the pathway of the 20mg zinc sulfate from ingestion and gut absorption to circulation, where it travels systemically bound to albumin. Crucially, it highlights the process of active secretion, where the zinc is actively pumped from the blood into the saliva and gingival crevicular fluid (GCF) via specialized ZnT transporters in the salivary glands. This physiological loop creates a zinc wash effect, ensuring that the oral tissues are continuously bathed in a zinc-rich fluid. The figure emphasizes that this delivery system creates a sustained therapeutic concentration that persists independent of oral hygiene habits, providing 24-hour protection against bacterial recolonization. Figure 6 serves as a comprehensive visual synthesis of the study's biological adjuvant theory. It integrates the structural repair of the host barrier with the direct biochemical inhibition of the pathogen, framed within a systemic delivery cycle. This schematic effectively argues that in stunted children, mechanical therapy (SRP) is insufficient because it addresses only the symptom (plaque) without correcting the underlying leaky gum pathology. The zinc shield mechanism demonstrates that systemic zinc supplementation provides the missing biological support necessary to break the cycle of reinfection, transforming the oral cavity from an open culture medium into a defended, resilient ecosystem. This mechanistic understanding elevates zinc from a simple dietary supplement to a sophisticated therapeutic agent capable of orchestrating a profound ecological rearrangement in the oral cavity.[15]

The results observed in the Control group challenge the traditional dogma that cleaner is better in immunocompromised patients. We observed a paradoxical increase in bacterial abundance post-treatment, specifically within the *Firmicutes* phylum, dominated by *Streptococcus* and *Veillonella*. This phenomenon aligns with the ecological theory of secondary succession. When the mature, dysbiotic biofilm is mechanically stripped away via scaling and root planing, the procedure exposes the nutrient-rich gingival crevicular fluid and the pellicle-coated tooth surface.[16] In a healthy host, the immediate immune response involves the secretion of salivary antimicrobial peptides, including defensins and histatins, as well as secretory IgA, which modulate the recolonization of this surface and select for benign commensals.

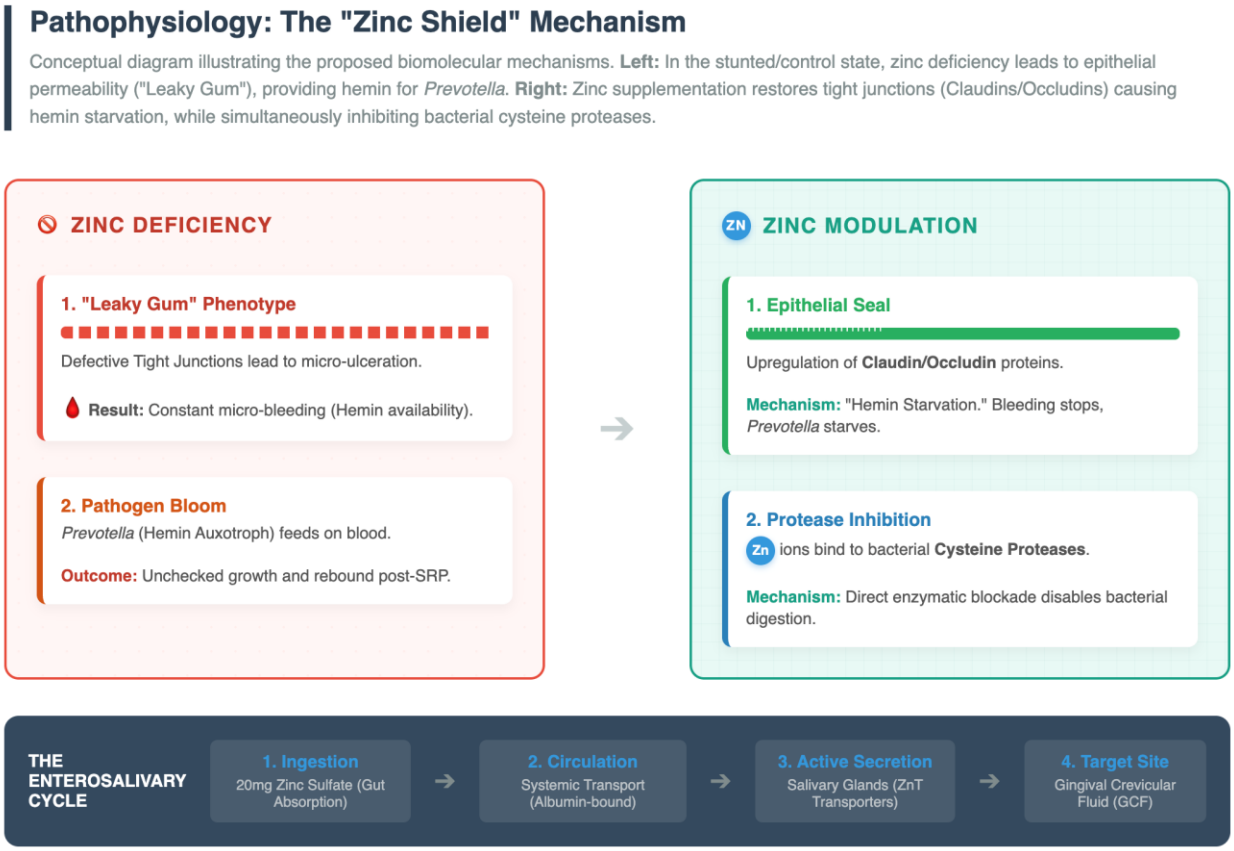


Figure 6. Pathophysiology zinc shield mechanism.

However, stunted children exhibit a phenotype of a compromised mucosal barrier. Chronic zinc deficiency impairs the synthesis of these salivary proteins and weakens the junctional epithelium. Consequently, the oral cavity of the stunted child acts as an open, unprotected culture medium. The rapid doubling time of pioneer colonizers such as *Streptococcus* species allows them to exploit this unchecked environment immediately after SRP. They adhere to the pellicle via antigen I/II polypeptides, and *Veillonella* species subsequently co-aggregate by metabolizing the lactate produced by *streptococci.*[17] The significant increase in these genera in the Control Group confirms that without biological support, mechanical therapy in stunted children merely resets the clock for biofilm accumulation rather than resolving the dysbiosis. This dysbiotic resilience explains why gingivitis in malnourished children is often recalcitrant and prone to rapid recurrence.

The most striking and clinically significant finding of this study is the complete suppression of *Prevotella* to undetectable levels in the zinc-supplemented group. *Prevotella* species, particularly *Prevotella intermedia* and *Prevotella nigrescens*, are classified as "orange complex" bacteria. They act as critical ecological bridges, facilitating the succession from early bacterial colonizers to the highly pathogenic red complex organisms such as *Porphyromonas gingivalis*. They are strongly associated with the onset of gingival bleeding and the progression of inflammation. The eradication of *Prevotella* by zinc likely involves a tripartite synergistic mechanism involving hemin starvation, protease inhibition, and oxidative stress. *Prevotella* species are auxotrophic for hemin; they lack the enzymatic pathway to synthesize their own iron and obligately rely on environmental blood (specifically heme) for survival. This is why they thrive in inflamed, bleeding gingival pockets.[18] Zinc is a

critical cofactor for the gene expression of Claudin-1 and Occludin, the tight junction proteins that seal the gingival epithelium. In the stunted child, zinc deficiency leads to leaky gum syndrome, where the epithelium is permeable and prone to micro-ulceration and bleeding. By supplementing with 20 mg of zinc, we hypothesize that the intervention upregulated these junctional proteins, accelerated epithelial healing, and, crucially, stopped the micro-bleeding. By sealing the tissue, zinc effectively cut off the hemin supply line. Without a source of blood, the hemin-dependent *Prevotella* starved to death.

Prevotella species rely heavily on cysteine proteases to degrade host proteins, including immunoglobulins and collagen, to acquire peptides for nutrition. These proteases are functionally similar to the gingipains found in *P. gingivalis*. Zinc is a known potent, non-competitive inhibitor of cysteine proteases. Chemically, the zinc ion binds to the active site thiol groups of these enzymes, effectively jamming the catalytic machinery. By neutralizing the proteolytic activity, zinc prevents *Prevotella* from digesting host tissues, thereby disabling their ability to feed and replicate. This direct enzymatic inhibition represents a pharmacological mode of action distinct from the host immune response. As strict anaerobes or facultative anaerobes, *Prevotella* species are highly sensitive to oxidative stress. To survive in the aerated oral cavity, they rely on superoxide dismutase (SOD) enzymes, which often require manganese as a cofactor. Paradoxically, at high localized concentrations in the gingival crevice—facilitated by the supplementation—zinc ions can competitively displace manganese in the bacterial transport systems (specifically the MntH transporter). This phenomenon, known as metal toxicity, deprives the bacteria of the manganese required for their antioxidant defense. Without functional SOD, *Prevotella* becomes vulnerable to the oxidative burst produced by host neutrophils, leading to rapid bacterial cell death.[19]

A critical question regarding oral supplements is the mechanism of delivery: how does a pill swallowed into the gut affect bacteria in the gingival sulcus? We propose the enterosalivary cycle as the primary delivery vehicle. Zinc absorbed in the small intestine is transported systemically bound to albumin. It is then actively sequestered and secreted into the saliva and gingival crevicular fluid (GCF) via ZnT (Zinc Transporter) proteins located in the acinar cells of the salivary glands. This creates a sustained, high-concentration zinc wash over the oral tissues, occurring continuously and independent of oral hygiene habits. This systemic recirculation ensures that the gingival crevice is constantly bathed in zinc-rich fluid, maintaining the inhibitory pressure on pathogenic bacteria even hours after the capsule is ingested. Furthermore, the significant reduction of the *Proteobacteria* phylum in the zinc group is of high systemic relevance. *Proteobacteria* are predominantly Gram-negative organisms possessing lipopolysaccharide (LPS) in their outer cell walls. In the context of stunting, the leaky gut and leaky gum phenomena allow these endotoxins to enter systemic circulation, contributing to chronic systemic inflammation, which further stunts growth via the suppression of Insulin-like growth factor 1 (IGF-1). Zinc has been shown to bind directly to the lipid A component of LPS, neutralizing its biological activity and preventing it from triggering toll-like receptor 4 (TLR4) on host immune cells. By reducing the absolute abundance of *Proteobacteria* by over 50%, zinc supplementation reduces the total Gram-negative burden in the oral cavity. This suggests that the intervention does not just treat the mouth; it likely reduces the total systemic inflammatory load, breaking the vicious cycle where inflammation inhibits the absorption of nutrients, which in turn exacerbates stunting.

Beyond direct toxicity and nutrient starvation, zinc likely modulates the behavior of the biofilm through interference with quorum sensing (QS). Bacterial recolonization and biofilm maturation are coordinated by chemical signaling molecules known as autoinducers. Zinc ions have been observed to interfere with the binding of these autoinducers to their receptors, such as the LuxR-type receptors found

in many Gram-negative bacteria. By jamming the communication lines between bacteria, zinc prevents the coordinated formation of the biofilm matrix. This keeps the bacteria in a planktonic, or free-floating, state where they are more easily cleared by salivary flow and swallowing, explaining the stabilization of pioneer colonizers like *Streptococcus* and *Veillonella* in the Zinc group.[20]

5. Conclusion

The findings of this study redefine the role of zinc in pediatric periodontology. In stunted children, the oral environment is not merely dirty; it is ecologically broken. Standard mechanical therapy, while necessary, is insufficient to repair this ecosystem and often leads to rapid bacterial rebound due to the compromised status of the host's mucosal barrier. Systemic zinc supplementation acts as a crucial biological scaffold. It does not simply support immunity; it actively reshapes the microbiome through a multi-targeted approach. By selectively eradicating *Prevotella*, the critical bridge organism for periodontal destruction, zinc halts the progression of dysbiosis. Suppressing the *Proteobacteria* load reduces the Gram-negative endotoxin burden that contributes to systemic inflammation. By stabilizing the *Firmicutes* population, it prevents the chaotic post-treatment bloom of pioneer species. Consequently, zinc supplementation should be elevated from a nutritional suggestion to a strongly recommended therapeutic adjuvant in the comprehensive management of gingivitis for stunted and nutritionally vulnerable children. This intervention offers a low-cost, high-impact strategy to restore the host-microbe equilibrium and protect the oral health of the most vulnerable pediatric populations.

6. References

1. Otxandoregi-Ieregi U, Spiridon A, Aleksza D, Escudero-Martinez C, Woebken D, George TS, et al. Unraveling zinc acquisition strategies in barley: the role of phytosiderophore exudation and root-associated microbiome. 2025.
2. Adams SE, Cawley AK, Arnold D, Hoptroff MJ, Slomka V, Matheson JR, et al. A randomised, double-blind clinical study into the effect of zinc citrate trihydrate toothpaste on oral plaque microbiome ecology and function. Sci Rep. 2025; 15(1): 8136.
3. da Silva CA, Bentin LAT, Dias CP, Callegari MA, Facina VB, Dias FTF, et al. Impact of zinc oxide, benzoic acid and probiotics on the performance and cecal microbiota of piglets. Anim Microbiome. 2021; 3(1): 86.
4. Ekhlas D, Cobo Díaz JF, Cabrera-Rubio R, Alexa E, Ortiz Sanjuán JM, Garcia Manzanilla E, et al. Metagenomic comparison of the faecal and environmental resistome on Irish commercial pig farms with and without zinc oxide and antimicrobial usage. Anim Microbiome. 2023; 5(1): 62.
5. Ortiz Sanjuán JM, Argüello H, Cabrera-Rubio R, Crispie F, Cotter PD, Garrido JJ, et al. Effects of removing in-feed antibiotics and zinc oxide on the taxonomy and functionality of the microbiota in post weaning pigs. Anim Microbiome. 2024; 6(1): 18.
6. Drake MJ, Daniel SG, Baker LD, Indugu N, Bittinger K, Dickens C, et al. Effects of dietary zinc on the gut microbiome and resistome of the gestating cow and neonatal calf. Anim Microbiome. 2024; 6(1): 39.
7. Drake MJ, Pierdon M, DeMers G, Daniel SG, Bittinger K, Redding LE. The effect of dietary zinc on the microbiome and resistome of the gestating sow and neonatal piglets. Anim Microbiome. 2025; 7(1): 71.
8. Al-Kamel A, Baraniya D, Al-Hajj WA, Halboub E, Abdulrab S, Chen T, et al. Subgingival microbiome of experimental gingivitis: shifts associated with the use of chlorhexidine and N-acetyl cysteine mouthwashes. J Oral Microbiol. 2019; 11(1): 1608141.
9. Huang S, He T, Yue F, Xu X, Wang L, Zhu P, et al. Longitudinal multi-omics and microbiome meta-analysis identify an

asymptomatic gingival state that links gingivitis, periodontitis, and aging. MBio. 2021; 12(2).

10. Scannapieco FA, Dongari-Bagtzoglou A. Dysbiosis revisited: Understanding the role of the oral microbiome in the pathogenesis of gingivitis and periodontitis: a critical assessment. J Periodontol. 2021; 92(8): 1071–8.
11. Wirth R, Maróti G, Lipták L, Mester M, Al Ayoubi A, Pap B, et al. Microbiomes in supragingival biofilms and saliva of adolescents with gingivitis and gingival health. Oral Dis. 2022; 28(7): 2000–14.
12. Vishnu RA, Alamelu S, Arun KV, Sujitha P, Ganesh PR. Comparative evaluation of subgingival microbiome in healthy periodontium and gingivitis using next-generation sequencing technology: a case-control study. J Indian Soc Periodontol. 2022; 26(3): 224–9.
13. Iniesta M, Chamorro C, Ambrosio N, Marín MJ, Sanz M, Herrera D. Subgingival microbiome in periodontal health, gingivitis and different stages of periodontitis. J Clin Periodontol. 2023; 50(7): 905–20.
14. Ningsih DS, Idroes R, Bachtiar BM, Khairan K, Tallei TE, Kemala P, et al. Clinical and oral microbiome pattern of halitosis patients with periodontitis and gingivitis. Narra J. 2023; 3(2): e163.
15. Blufstein A, Pejcic N, Spettel K, Hausmann B, Seki D, Ertekin T, et al. Salivary microbiome and MRP-8/14 levels in children with gingivitis, healthy children, and their mothers. J Periodontol. 2024; 95(11): 1035–47.
16. Ge Y, Bamashmous S, Mancinelli-Lyle D, Zadeh M, Mohamadzadeh M, Kotsakis GA. Interdental oral hygiene interventions elicit varying compositional microbiome changes in naturally occurring gingivitis: Secondary data analysis from a clinical trial. J Clin Periodontol. 2024; 51(3): 309–18.
17. Manzoor M, Leskelä J, Pietiäinen M, Martinez-Majander N, Könönen E, Niiranen T, et al. Shotgun metagenomic analysis of the oral microbiome in gingivitis: a nested case-control study. J Oral Microbiol. 2024; 16(1): 2330867.
18. Yang H, Ma Y, Gao H, Xie X, Wang H, Li X, et al. Supragingival microbiome variations and the influence of *Candida albicans* in adolescent orthodontic patients with gingivitis. J Oral Microbiol. 2024; 16(1): 2366056.
19. Iniesta M, Vasconcelos V, Sanz M, Herrera D. Supra- and subgingival microbiome in gingivitis and impact of biofilm control: a comprehensive review. Antibiotics (Basel). 2024; 13(6): 571.
20. van der Ploeg GR, Brandt BW, Keijser BJF, van der Veen MH, Volgenant CMC, Zaura E, et al. Multi-way modelling of oral microbial dynamics and host-microbiome interactions during induced gingivitis. NPJ Biofilms Microbiomes. 2024; 10(1): 89.