## Title

Diet texture, not fiber content, modulates host and microbial phenotypes linked to periodontitis



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Diet texture, not fiber content, modulates host and microbial phenotypes linked to periodontitis

by
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"*Find what it is that you love to do and take it to heart with the greatest conviction."*

*-Sunita Ho*

# Diet texture, not fiber content, modulates host and microbial phenotypes linked to periodontitis

**Lea M. Sedghi**

## Abstract

The Western diet, characterized by high consumption of refined, processed grains, has been epidemiologically associated with elevated periodontal disease risk, yet the mechanisms underlying this relationship remain incompletely understood. Grain refinement simultaneously removes dietary fiber and reduces food texture, producing a soft, rapidly fermentable substrate that may promote oral biofilm dysbiosis through two distinct but potentially synergistic pathways: augmented saccharide substrate availability to saccharolytic pioneer species, and loss of the masticatory mechanical challenge that limits biofilm maturation and sustains homeostatic gingival immune signaling. Whether the protective effects of whole grain consumption are attributable to its fiber content, its structural and textural properties, or both has not been experimentally resolved.

The present thesis employed a multi-diet murine experimental framework to dissociate the contributions of dietary texture and fiber content to periodontal host-microbe homeostasis. In Chapter 2, Sprague-Dawley rats were fed hard or soft nutritionally matched diets, and dental

biofilm abundance was assessed by scanning electron microscopy. In parallel, coarse- and fine-particle wheat digests were used to grow human saliva-derived oral biofilms *in vitro*, with biofilm architecture characterized by scanning electron microscopy and growth kinetics quantified by 24-hour spectrophotometry. In Chapters 3 and 4, BALB/cByJ mice were assigned to one of five dietary regimens: a Standard Diet (SD), Whole Grain (WG), Processed Grain (PG), Fiber Bar (FB), or Placebo Bar (PB). Within each dietary group, animals were further divided into sham-inoculated and polymicrobial-inoculated subgroups receiving a topical oral lavage of *Porphyromonas gingivalis*, *Treponema denticola*, *Tannerella forsythia*, and *Fusobacterium nucleatum*. Gingival inflammatory cytokine profiles (TNF-α, IL-1β, IL-6, IL-23, IL-17A, IL-10) were quantified by multiplex assay, alveolar bone loss was measured by micro-computed tomography, and oral microbial community composition was characterized by 16S rRNA V4 amplicon sequencing with multivariate and differential abundance analyses.

Across all experimental chapters, dietary texture emerged as the dominant modifier of periodontal outcomes, with diet consistently explaining more variance than polymicrobial inoculation status. In Chapter 2, hard-diet animals accumulated significantly less dental biofilm than soft-diet counterparts, and fine-particle grain digests supported greater bacterial growth kinetics and larger biofilm surface areas than coarse-particle digests across all digestion intervals. In Chapter 3, WG-fed animals exhibited significantly lower alveolar bone loss and reduced gingival IL-17A levels compared to PG- and FB-fed animals, while no significant differences were observed between FB and PB groups differing only in fiber supplementation. A positive correlation between gingival IL-17A and total alveolar bone loss across all dietary groups implicated IL-17A as a mechanistic intermediate linking dietary texture to periodontal bone outcomes. In Chapter 4, the most statistically robust differences in oral microbial community composition were observed

between WG and FB groups, diets differing in fiber form, with WG selectively enriching *Lactobacillus* and processed grain and fiber bar diets enriching *Enterococcus* and *Staphylococcus*. Among inoculated animals, processed grain diet uniquely supported enrichment of *Fusobacterium* and *Prevotella*, suggesting that grain refinement creates an ecologically permissive oral environment for periodontal pathogen colonization. Collectively, these findings identify dietary texture—independent of fiber content—as a dominant and mechanistically grounded determinant of periodontal host–microbe homeostasis, with implications for dietary prevention strategies, pediatric oral health policy, and the reduction of the substantial individual and societal costs of periodontal disease and edentulism.

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## Chapter 1: Introduction

### *The Periodontal Complex: Anatomy, Function, and Clinical Significance*

The periodontium provides attachment and support for the dentition within the alveolar processes of the jaws. It comprises four interrelated tissues: the gingiva, periodontal ligament (PDL), cementum, and alveolar bone proper. These tissues act in concert to anchor the tooth root to the surrounding osseous socket while simultaneously absorbing and distributing occlusal forces generated during mastication. The gingiva, as the most superficial component, serves as the primary physical barrier separating the oral environment from the underlying supporting apparatus. Its junctional epithelium and sulcular epithelium form a critical interface at the dentogingival junction, where the transition from oral mucosal epithelium to a non-keratinized, highly permeable epithelium renders this zone particularly vulnerable to microbial colonization and immune challenge.[1,2]

The periodontal ligament is a dense fibrous connective tissue that occupies the space between the tooth root cementum and the inner surface of the alveolar socket. It performs biomechanical, sensory, and nutritive functions while also maintaining a population of progenitor cells capable of contributing to periodontal regeneration following injury. The alveolar bone undergoes continuous remodeling in response to both physiological occlusal stimuli and pathological inflammatory signals. The balance between osteoblastic bone formation and osteoclastic resorption is tightly regulated under homeostatic conditions; however, sustained periodontal inflammation disrupts this equilibrium, shifting the system toward net bone loss. Such alveolar bone destruction is the hallmark of periodontitis and the principal determinant of tooth loss associated with advanced disease.[1,2,3]

The clinical importance of periodontal health extends considerably beyond oral function. Epidemiological evidence has established robust associations between periodontitis and systemic conditions including cardiovascular disease, type 2 diabetes mellitus, adverse pregnancy outcomes, rheumatoid arthritis, chronic kidney disease, and Alzheimer's disease.[4,5,6] These associations are supported by shared pathophysiological mechanisms, including systemic dissemination of pro-inflammatory cytokines, bacteremia from periodontal pathogens, and bidirectional amplification of inflammatory cascades. The mouth, as a portal to the systemic circulation and immunological priming, is therefore not an isolated anatomical domain but rather an immunologically active gateway whose homeostatic status carries consequences for whole-body health.[4,5]

***Microbial Biofilm Formation and the Dental Plaque Ecosystem***

Unlike the mucosal surfaces of the gastrointestinal or respiratory tracts, dental hard tissues are non-shedding surfaces that persist in the oral environment throughout the lifetime of the dentition. This structural permanence creates a uniquely favorable substrate for microbial colonization. Dental surface layers do not intrinsically shed to prevent excess biofilm accumulation, and the complex geography of dental surfaces, such as supragingival crevices and fissures, as well as the subgingival sulcus provides microenvironments with variable oxygen tension, pH, nutrient availability, and vulnerability to shear forces. Each site serves as a unique microenvironment that in turn shapes the dental composition and metabolic activity of dental biofilm communities[7,8]

Dental biofilm assembly is a temporally organized, multi-stage process: primary colonization begins within minutes of tooth cleaning, as salivary glycoproteins adsorb onto the pellicle-coated enamel surface. Pioneer species, predominantly streptococcal taxa such as

*Streptococcus gordonii* and *Streptococcus sanguinis*, exploit surface adhesins to bind to specific receptors within this acquired pellicle. These early colonizers modify the local microenvironment through metabolic activity and extracellular matrix production, creating conditions conducive to the attachment of secondary colonizers. *Fusobacterium nucleatum* occupies a pivotal bridging role in this succession, possessing co-aggregation ligands that facilitate adhesion between early streptococcal colonizers and late-colonizing anaerobes.[7,8,9] Late-stage colonization by Gram-negative anaerobic species, including members of the red complex, including *Porphyromonas gingivalis*, *Treponema denticola*, and *Tannerella forsythia*, marks the transition toward a potentially dysbiotic biofilm state associated with periodontal disease pathogenesis.[10,11]

Within the mature biofilm, microbial species occupy chemically and spatially defined niches that reflect complex interspecies interdependencies. These include metabolic syntropies in which the metabolic byproducts of one species serve as growth substrates for another, as well as quorum-sensing-mediated communication, co-aggregation through surface adhesin-receptor interactions, and competitive antagonism via bacteriocin production.[7,8] The three-dimensional architecture of the biofilm, embedded within a self-produced extracellular polymeric matrix of polysaccharides, proteins, and extracellular DNA, confers marked resistance to antimicrobial agents and host immune effectors. Collectively, these emergent properties render the dental biofilm metabolically distinct from the individual microbes comprising it. The composition and metabolic state of the biofilm, rather than microbial burden alone, ultimately determine its pathogenic potential with respect to both caries and periodontal disease.[7,8,9]

### *The Environmental Plaque Hypothesis and Microbial Dysbiosis*

Classical interpretations of periodontal disease etiology are centered on disease induction by a set of pathogenic species, most prominently *Porphyromonas gingivalis*, *Treponema denticola*,

and *Tannerella forsythia*, which are necessary and sufficient agents in periodontal destruction. This notion has shaped research and therapeutics aimed at identifying and targeting specific periodontal pathogens. While these organisms remain clinically significant, more ecologically nuanced frameworks better accommodate the complexity of periodontal microbiology and the role of the dental biofilm as a community as it relates to states of periodontal pathology.[9,12]

The ecological plaque hypothesis, and its more contemporary extension, the polymicrobial synergy and dysbiosis model, propose that periodontal disease is fundamentally a community-level ecological disturbance rather than simply a host-pathogen infection.[12,13] Under this framework, environmental perturbations, including changes in nutrient availability, oxygen tension, pH, and inflammatory mediators, alter the selective pressures operating within the biofilm ecosystem. These shifts enrich taxa that thrive under the altered conditions, ultimately incorporating proteolytic anaerobes, capable of exploiting host-derived inflammatory exudate, which induce periodontal destruction directly. The resulting dysbiotic community in turn generates sufficient inflammatory challenge to perpetuate the cycle of tissue destruction and ecological shift.[12,13,14]

The environmental plaque hypothesis specifically implicates diet as a central environmental determinant of biofilm composition.[12] Dietary substrates, particularly fermentable carbohydrates, determine the saccharolytic metabolic activity of the supragingival biofilm, the resulting local acid pH, and the selective advantage of acid-tolerant organisms. Little attention has turned to the potential environmental role of dietary fiber in driving changes in biofilm composition. By contrast, dietary fiber is not metabolized within the oral cavity and thus modulates the physical environment of the dentogingival surfaces through masticatory mechanical challenge. Diet-mediated ecological pressures operate continuously throughout the lifetime of the host and

fluctuate continuously, thus rendering dietary patterns among both the most central and dynamic environmental modifiers of oral microbial community structure. Understanding how specific dietary attributes, including fiber content and dietary texture, influence biofilm ecology therefore has fundamental implications for understanding the etiology and prevention of periodontal disease.[15,16,17]

### *Periodontal Pathogens and the Host Inflammatory Response*

Red complex organisms including *Porphyromonas gingivalis*, *Treponema denticola*, and *Tannerella forsythia* represent some of the most extensively characterized members of the dysbiotic periodontal microbiota.[10,11] These organisms possess a diverse repertoire of virulence factors that enable colonization of the subgingival niche, subversion of innate immune signaling, and exploitation of host tissue catabolism as a metabolic resource. *P. gingivalis* has been characterized as a "keystone pathogen" capable of remodeling the wider microbial community from a commensal to a dysbiotic state, disproportionate to its numerical abundance within the biofilm.[14] Its gingipain cysteine proteases degrade complement components, cleave cytokine receptors, and disrupt toll-like receptor signaling, enabling *P. gingivalis* to impair innate immune surveillance while simultaneously promoting a nutrient-rich inflammatory environment that benefits the broader dysbiotic community.[14]

The host inflammatory response to the dysbiotic biofilm is mediated through a cascade of innate and adaptive immune mechanisms. Pattern recognition receptors on gingival epithelial cells and resident immune cells detect conserved microbial-associated molecular patterns, triggering the release of pro-inflammatory cytokines including interleukin-1β (IL-1β), interleukin-6 (IL-6), interleukin-8 (IL-8), and tumor necrosis factor-α (TNF-α).[1,2] These cytokines recruit professional immune effectors, neutrophils, macrophages, and later T and B lymphocytes, to the gingival

tissues. While this immune mobilization is essential for limiting microbial invasion, its persistence in the face of ongoing microbial challenge becomes pathological. Chronic neutrophil-mediated oxidative burst and matrix metalloproteinase release degrade the connective tissue matrix of the gingiva and PDL, while osteoclastogenic signals, particularly receptor activator of nuclear factor κB ligand (RANKL) generated by activated T cells, drive net alveolar bone resorption.[1,2,3]

The irreversibility of this inflammatory cascade, once established, distinguishes periodontitis from gingivitis. Gingivitis represents a reversible inflammatory response confined to the gingival soft tissues; resolution follows effective biofilm disruption. Periodontitis, defined by attachment loss and alveolar bone destruction, reflects a point of no return in which sustained inflammation has structurally compromised the periodontal apparatus. The transition from gingivitis to periodontitis is not inevitable, it is modulated by host immune competence, genetic susceptibility, and critically, by environmental factors including diet, but once periodontitis is established, lost periodontal support cannot be fully regenerated without surgical intervention.[1,2,3]

***Diet and Periodontal Disease: Epidemiological and Clinical Evidence***

As human diets have changed throughout history, so too has the relationship between diet and oral disease. Study of ancient calcified dental plaque have provided compelling evidence that the shift from hunter-gatherer to agricultural subsistence represented a major inflection point in the oral microbiome and in the incidence of oral pathology. Analysis of ancient dental calculus samples spanning the Mesolithic through the medieval period demonstrates a progressive enrichment in cariogenic and periodontitis-associated bacterial taxa coincident with the adoption of agricultural grain consumption and the progressive refinement and processing of dietary carbohydrates. The Mesolithic dentition, characterized by a diverse and predominantly non-saccharolytic microbiota, contrasts markedly with post-Neolithic samples showing expansion of

acid-producing streptococci, depletion of health-associated commensals, and reduced overall phylogenetic diversity. These paleomicrobiological findings are complemented by skeletal and dental morphological studies demonstrating increased prevalence of alveolar bone loss and carious lesions in Neolithic populations relative to their Mesolithic predecessors.[18]

Contemporary cross-sectional and observational studies corroborate these historical trends. Research among extant populations occupying traditional dietary patterns, including the Hadza hunter-gatherers of Tanzania, demonstrates dramatically superior oral health profiles relative to neighboring populations consuming Westernized, agriculturally based diets. Hadza females occupying a "bush" lifestyle characterized by high dietary fiber intake, minimal processed carbohydrate consumption, and high masticatory mechanical demand show significantly lower rates of periodontal disease, dental caries, and malocclusion compared to villager counterparts exposed to market-based foods. These findings suggest that the oral health benefits of pre-agricultural diet are not attributable to genetic or ethnic differences but rather to the dietary and mechanical properties of the food consumed.[19,20]

Prospective and interventional clinical data further support a causal role for dietary composition in periodontal health. Analysis of NHANES data spanning 2009–2012 demonstrated a significant inverse relationship between dietary fiber and whole grain consumption and the prevalence of moderate-to-severe periodontal disease, with individuals in the lowest quartile of fiber intake exhibiting substantially greater periodontal burden.[21] Dietary intervention studies have shown that substitution of a typical Western diet with an anti-inflammatory dietary pattern, characterized by high fiber intake, reduced refined carbohydrates, increased omega-3 fatty acids, and elevated dietary antioxidants, significantly reduces gingival bleeding on probing even without changes in mechanical oral hygiene practice.[22,23] Critically, a high-fiber, low-fat dietary

intervention reduced periodontal pocket probing depth, bleeding on probing, and clinical attachment loss in at-risk subjects, with these improvements persisting beyond the active dietary intervention period.[22] Taken together, these clinical observations identify diet not merely as a modifiable risk factor for periodontal disease, but as a potentially therapeutic lever with the capacity to alter the trajectory of periodontal inflammation.

### *Dietary Fiber: Mechanistic Roles in Oral Health*

Dietary fiber encompasses a heterogeneous group of plant-derived polysaccharides and oligosaccharides that resist digestion by mammalian digestive enzymes. These compounds can be broadly classified by their physical state (soluble vs. insoluble), degree of fermentability, and structural source (cereal, legume, fruit, vegetable). In the context of oral health, dietary fiber exerts effects through at least two distinct and partially independent mechanisms: a metabolic pathway mediated by the fermentability and saccharolytic accessibility of fiber-derived substrates to the oral microbiota, and a physical-mechanical pathway mediated by the masticatory forces and thus the direct surface abrasion generated by fibrous, textured food structures.[15,16,24]

The metabolic effects of dietary fiber on the oral microbiome are complex and bidirectional. Insoluble cellulosic fibers, such as those abundant in whole grains and vegetables, contribute little direct substrate for oral microbial fermentation and may instead favor health-associated commensal taxa by decreasing the relative availability of rapidly fermentable carbohydrates.[15,25] The replacement of refined carbohydrates with fiber-rich foods also reduces the bioavailability of starch to salivary amylase, the rate-limiting step in the conversion of starch to fermentable glucose in the supragingival plaque, thereby limiting the saccharide supply available to dysbiotic oral taxa.[15,16,26]

The physical-mechanical effects of dietary texture have received little attention with relevance to oral health yet are arguably as important as metabolic effects in the context of periodontal disease. Fibrous, hard, or textured food structures impose mechanical shear and compressive forces on dental and periodontal tissues during mastication. These forces have been shown to reduce established biofilm through direct abrasion of dental surfaces and through the stimulation of increased salivary flow, which provides antimicrobial peptides, immunoglobulins, bicarbonate buffering capacity, and pellicle proteins.[19,20,27] Beyond biofilm disruption, masticatory forces transmitted to the periodontium via the PDL generate a distinct, mechanically conditioned inflammatory microenvironment in gingival tissues that is qualitatively different from the pathological inflammation driven by microbial challenge. This force-driven gingival response appears to confer a degree of tissue conditioning and barrier enhancement that is absent when dietary texture is removed, an effect that may explain why populations consuming soft, processed diets are disproportionately vulnerable to periodontal disease even when oral hygiene is nominally maintained.[19,20]

### *Grain Refinement and Processed Carbohydrate Consumption*

Grain refinement is the dominant food processing operation underlying the structural and nutritional transformation of cereal grains in the Western food supply. The milling process removes the bran and germ—the outer fibrous layer and the nutrient-dense embryonic core, respectively—to isolate the starchy endosperm. This process yields a product with dramatically reduced dietary fiber content, altered macronutrient composition, and fundamentally modified physical structure. The bran, which in the intact grain mechanically encapsulates starch granules within a fibrous matrix, is the primary structural component responsible for retarding amylolytic starch digestion. Its removal exposes starch granules previously protected by fibrous

encapsulation, markedly increasing the exposed surface area-to-volume ratio available for salivary amylase attack.[26,28,29] More rapid and complete hydrolysis of refined starch by salivary amylase generates elevated glucose and maltose concentrations in the oral cavity, which provides a substrate for saccharolytic organisms including streptococci, lactobacilli, and other species comprising primary layers of the dental biofilm, that ultimately favors the outgrowth of proteolytic anaerobic species associated with periodontal pathogenesis.[12,13, 26]

The structural consequences of grain refinement extend beyond nutritional composition. In addition to removing fiber, milling disrupts the grain architecture, yielding a product with a soft, homogeneous texture that exerts minimal masticatory challenge.[26,28,29] This reduction in dietary texture has implications for both the mechanical disruption of dental biofilm and for the stimulation of force-driven protective mechanisms in gingival tissues, as described above. The confluence of increased fermentable substrate availability and reduced dietary mechanical challenge may therefore represent a dual dietary insult to periodontal homeostasis, one that is largely invisible when periodontal risk factors are assessed in terms of isolated nutrients rather than the integrated structural and nutritional properties of the food matrix.[15,16]

### *Dietary Fiber and the Murine Oral Microbiome*

The foundational experimental basis for the present thesis was established by prior work from our group demonstrating that dietary fiber exerts a significant and measurable effect on the composition of the murine dentogingival microbiome in which mice consuming a diet supplemented with dietary fiber in conjunction with sucrose (Fiber + Sugar) demonstrated markedly increased oral microbial diversity relative to animals consuming sucrose alone.[25] Genus-level analyses revealed that fiber supplementation was associated with increased bacterial diversity that was lost in the presence of sugar alone, thereby suggesting that dietary fiber modifies the

microbial composition of the oral cavity.[25] These findings implicated dietary fiber as an ecologically significant modifier of oral microbial community composition and raised the possibility that the effects of fiber are exerted at least in part through physical-mechanical, rather than purely metabolic, mechanisms, given that the fiber used in those experiments (lignin-based insoluble fiber) does not readily serve as a fermentable oral microbial substrate.[25,30]

These foundational findings, together with the epidemiological and clinical evidence linking refined carbohydrate consumption to periodontal disease, motivated the systematic multi-diet experimental design of the present thesis. A critical gap remained, however, in the mechanistic understanding of how dietary texture and fiber content independently and interactively influence the host-microbe dynamics that determine periodontal disease susceptibility. Specifically, it was unclear whether the beneficial effects of whole grain or high-fiber diets on periodontal outcomes were attributable to the fiber content, to the structural and textural properties of intact dietary fiber, to the reduction in refined carbohydrate intake that fiber-rich diets entail, or to some combination of these factors.[15,16,25] Disentangling these mechanisms requires experimental designs that can independently vary dietary texture and fiber content while controlling for macronutrient composition, an approach that motivates the experimental framework described in the present thesis.

***Experimental Rationale and Hypothesis***

The present thesis investigates the mechanistic pathways by which dietary texture and fiber content regulate periodontal host-microbe homeostasis. To address this question, five dietary regimens were developed and tested in a murine model of experimental periodontitis:[31] a Standard Diet (SD) serving as a physiological reference, a Whole Grain (WG) diet and a Processed Grain (PG) diet to investigate the effects of grain refinement, and a Fiber Bar (FB) diet paired with a

Placebo Bar (PB) diet to determine the independent contribution of fiber supplementation in the absence of grain texture. By varying dietary texture and fiber content as distinct experimental parameters, and by testing all regimens both in the presence and absence of experimental polymicrobial periodontal challenge, this design enables a controlled dissociation of the mechanisms through which dietary properties influence alveolar bone morphology, gingival inflammatory cytokine signaling, and oral microbial community structure.[15,16,25]

The central hypothesis of this thesis is that dietary texture—the physical-mechanical property of food determined primarily by grain integrity and fiber structure—is the dominant dietary modifier of periodontal host-microbe homeostasis, and that this textural effect is not fully recapitulated by fiber supplementation alone in the absence of food texture. This hypothesis is grounded in the mechanistic arguments outlined above: the capacity of food texture to mechanically disrupt dental biofilm and provoke a force-conditioned protective inflammatory response in gingival tissues represents a fundamentally distinct biological pathway from the metabolic modulation of biofilm ecology by fiber fermentability.[15,16,19,20] The WG vs PG comparison directly tests the integrated effects of grain processing, which simultaneously removes fiber and reduces dietary texture, while the FB vs PB comparison isolates the contribution of fiber supplementation independent of textural properties.

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## Chapter 2: The impact of food particle size on biofilm architecture, abundance, and growth kinetics *in vitro.*

### Introduction

The emergence of periodontal disease (PD) corresponds to dietary shifts and changes in the composition of the oral microbiome associated with evolution of the Western diet,[1,2,3,4] which is predominantly comprised of refined, processed grains that today constitute nearly 50% of daily caloric intake.[1,5,6] Processing strips away the outer fibrous layer of grains to isolate the inner starch-rich endosperm.[7,8] In doing so, the integrity of the grain structure is disrupted, resulting in increased bioavailability of inner starch-rich components and decreased grain texture.[7,9,10,11] Diets high in processed grains are correlated with periodontal disease and other chronic inflammatory conditions.[12,13,14,15] However, a causative mechanism by which refined grain consumption promotes PD is poorly understood.

Oral digestion begins with enzymatic breakdown of carbohydrates by salivary amylase and mechanical processing by mastication.[16,17,18] Carbohydrate digestion is initiated via hydrolysis of starch by salivary amylase, which cleaves alpha-1,4 glycosidic linkages, yielding smaller saccharide moieties that can be utilized by early biofilm colonizers as well as by some periodontal pathogens.[19,20,21] Moreover, dentogingival surfaces lack an inherent shedding mechanism, thereby enabling mature biofilm formation in which successional microbial colonization induces changes in biofilm structure, composition, and activity that may ultimately facilitate a higher representation of periodontal pathogens.[22,23,24] As such, mechanical debridement is needed to limit biofilm formation.[25,26] Masticatory challenges provided by increased diet texture disrupt biofilm and are suggested to play a role in maintaining periodontal health.[27,28,29]

Processing increases starch bioavailability and its susceptibility to salivary amylase,[7,17,30] and may increase saccharide availability to bacteria in the oral cavity and encourage dysbiosis.[19,20,31] Moreover, as grain processing disrupts the integrity of the grain structure,[7,11] such changes may reduce masticatory forces, thus encouraging excess biofilm formation and resulting in microbial dysbiosis.[27,28] The objective of these preliminary studies was to investigate 1) if diet hardness impacts oral biofilm abundance *in vivo* and 2) if variation in food particle size induces changes in oral biofilm abundance. For objective 1, Sprague-Dawley rats were fed nutritionally matched hard- or soft-diets for 6 weeks. SEM images of murine dental surfaces revealed striking differences in biofilm abundance, in which exacerbated biofilm accumulation was observed in the soft diet group. For objective 2, biofilms supplemented with smaller (Fine) or larger (Coarse) wheat particle digests were grown *in vitro* and were analyzed using scanning electron microscopy (SEM) and growth curve analysis. Biofilms grown *in vitro* demonstrated significant variation in biofilm abundance and, surprisingly, biofilm architecture.

As microbial dysbiosis and biofilm accumulation are extremely relevant to the etiology of periodontal disease,[22,23,24] it is important to understand the mechanisms by which dietary structural properties control biofilm accumulation and shape microbial communities to advance dental disease prevention. Here, we demonstrate the first functional characterization of the influence of dietary particle size on microbial biofilm abundance and structure in the oral cavity.

## Results

### *Dietary hardness impacts oral biofilm loading in vivo.*

To gain insight as to how food hardness impacts oral biofilm abundance *in vivo*, 4-week old Sprague Dawley rats were fed one of two nutritionally equivalent foods: hard pellet food (Hard Food = HF; n=1) or soft powdered chow (Soft Food = SF; n=1) for 6 weeks; foods differed only in hardness (softer food was a powdered version of the pelleted chow). Right hemimandibles (n=1) were imaged with scanning electron microscopy. Sparse microbial biofilm was observed among the coarse (hard) diet group (**Fig. 2.1**), which demonstrated dental surfaces with abrasion indicative of mechanical challenge. In contrast, the fine (soft) diet group demonstrated significantly increased biofilm accumulation consistent with mature dental biofilm formation.

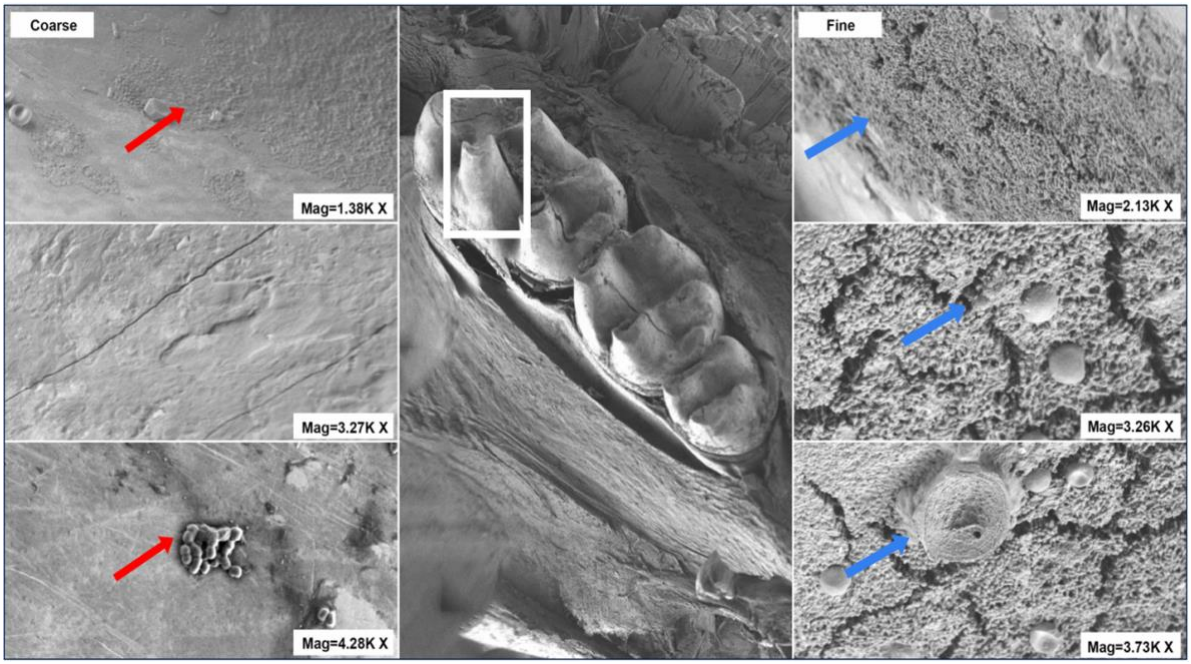


**Figure 2.1. Food hardness impacts dental biofilm abundance.** Dental surfaces of mice supplemented with coarse (n=1) or fine (n=1) diets reveal striking differences in biofilm abundance following 6-week treatment with coarse (left) and fine (right) diets. Arrows indicate bacterial biofilm observed in the coarse (red) and fine (blue) groups.

***Variation in food particle size drives changes in biofilm architecture.***

To determine the impact of degree of food processing on oral biofilms, an *in vitro* model was developed to capture the effect of grain processing on oral biofilm growth. Wheat berries were milled into either coarse or fine particles to represent unprocessed and processed grain, respectively (**Fig. 2.2**). As coarse grains retained the outer bran layer (fiber), inner starch components are less susceptible to enzymatic attack by salivary amylase compared to fine particles.[7,9,30]

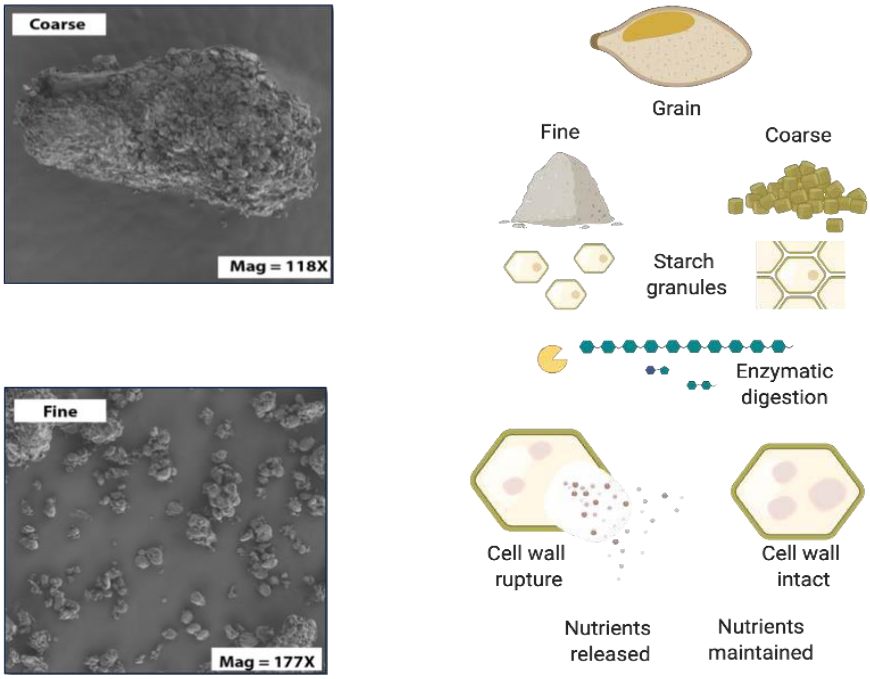


**Figure 2.2. Food particle size influences starch bioavailability.** SEM of Coarse and Fine grain particles demonstrates differences in surface area to volume ratio of starch granules (left). Starch granule surface area to volume ratio influences susceptibility to enzymatic attack and release of nutrients by salivary amylase (right).

Coarse or fine grain particles were then subjected to an *in vitro* oral model of processed and unprocessed grain consumption (**Fig. 2.3**): grain particles were placed in a sterile 1.5 mL Eppendorf tube, suspended in cell-free human saliva, and subjected to digestion by salivary amylase present in saliva for either 2.5-, 5-, 10-, or 20-min digestion intervals at 34.5°C. Grain digests were then collected by centrifugation and transfer of cell-free saliva and released saccharides to a clean Eppendorf tube. Samples were heat-shocked to halt amylase activity. To determine the subsequent impact of degree of grain digestion on bacterial biofilm growth, human saliva-derived oral biofilms were grown by adding cell-containing saliva to coarse or fine grain digest per well in 24-well plates (n=4). Biofilms were incubated under aerobic conditions at 37°C for 24 hours.

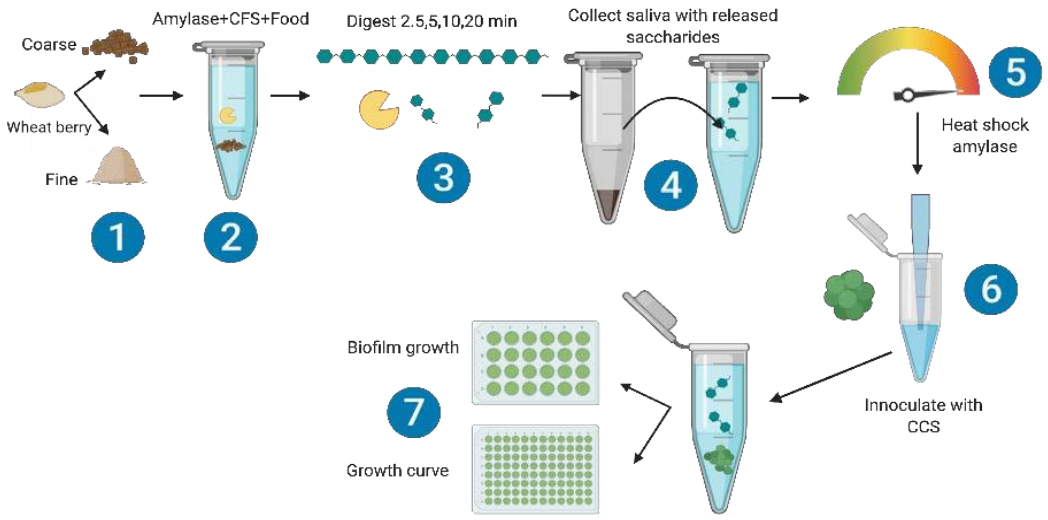


**Figure 2.3. *In vitro* model demonstrating the effect of grain particle size on digestion in the oral cavity.** 1) Wheat berries were milled into either coarse or fine grain particles. 2) Grain particles were added to 1mL of cell-free saliva (CFS) and 3) subjected to digestion by salivary amylase for 2.5-, 5-, 10-, or 20-minute intervals. 4) Saccharides released during starch digestion were separated from un-digested components via centrifugation. 5) CFS containing released saccharides was heat-shocked to halt further enzymatic digestion by salivary amylase. 6) CFS containing released saccharides was inoculated with oral microbes from healthy volunteers. 7) Oral bacteria were subjected to growth as biofilms and growth curves were captured to determine impact on bacterial growth kinetics.

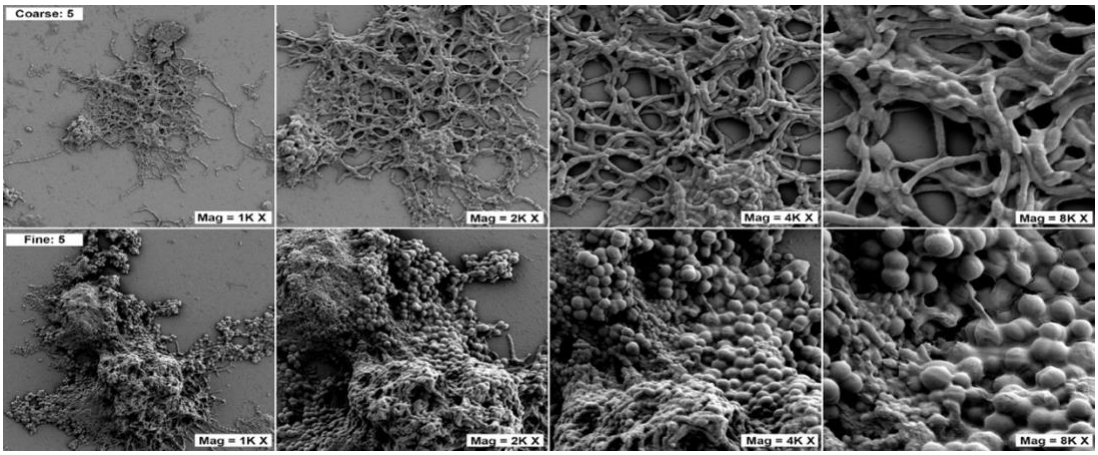


**Figure 2.4. Grain particle size impacts oral biofilm architecture.** Oral bacteria grown as biofilms in Coarse grain particle digest (top) and Fine grain particle digest (bottom). Coarse and Fine grains were subjected to enzymatic degradation by salivary amylase for 5-minute digestion interval. Coarse and Fine biofilms were imaged using SEM and demonstrated striking differences in biofilm structure.

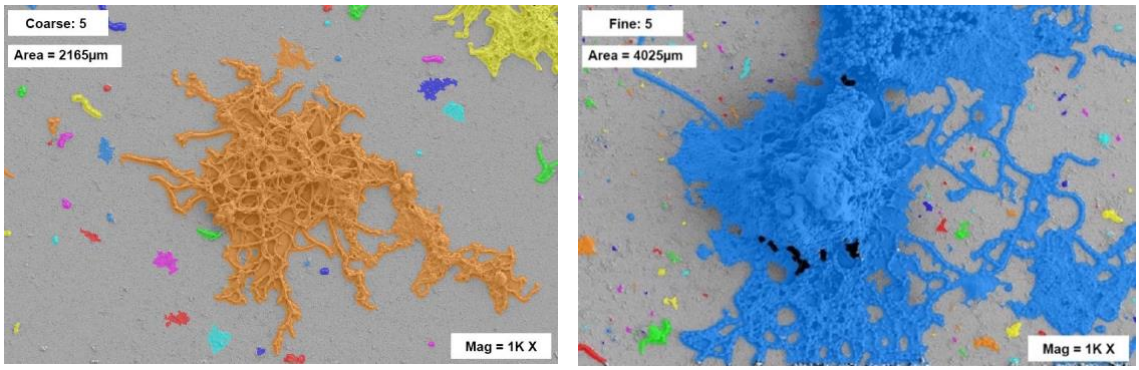


**Figure 2.5. Grain particle size impacts oral biofilm abundance.** Oral bacteria grown as biofilms in Coarse grain particle digest (top) and Fine grain particle digest (bottom). Coarse and Fine grains were subjected to enzymatic degradation by salivary amylase for 5-minute digestion interval. Coarse and Fine biofilms were imaged using SEM and surface area of biofilms was captured using AVIZO software. Biofilms grown in coarse grain digests (2165um) demonstrated decreased surface area compared to those grown in fine particle digests (4025um).

Following this, biofilms were imaged using scanning electron microscopy. Biofilms grown in coarse or fine grain digests demonstrated striking differences in biofilm morphology (**Fig. 2.4**). Because oral biofilm structure is reflective of microbial community composition and function,[22,23] these data suggested that grain particle size, representative of degree of processing, impacts composition and metabolic potential of oral biofilms. Biofilms in coarse grain digests also demonstrated decreased total surface area (2,164 μm) compared to those grown in fine grain digests (4,025 μm) (**Fig. 2.5**), suggestive of decreased substrate availability to oral bacteria compared to those grown in fine grain digests.

***Grain processing increases oral biofilm growth kinetics.***

To determine if grain processing impacts microbial growth kinetics, coarse and fine grain particle digests (subjected to digestion by salivary amylase for 2.5, 5, 10, or 20-minute digestion intervals) were used as growth media for oral bacteria and bacterial growth kinetics were then assessed via 24-hr spectrophotometer readings. Microbial growth among coarse and fine grain digests was then quantified and compared using area under the curve (n=4). Significantly greater growth potential was observed among bacteria grown in the fine grain digest across all digestion time intervals (n=4; Student's t-test; 2.5 min, $p$=0.021; 5.0 min, $p$=0.025; 10 min, $p$=0.034; 20 min, $p$=0.023) (**Fig. 2.6**), suggesting that grain processing alters nutrient availability and increases oral bacterial growth kinetics.

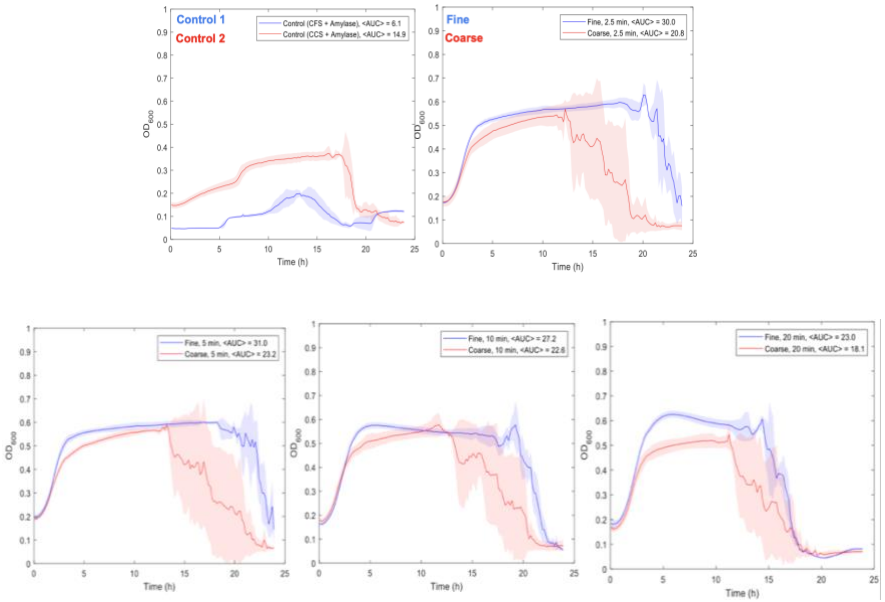


**Figure 2.6. Growth curve analysis of oral biofilms grown in either coarse or fine grain digests for 24 hours.** Biofilm optical density over time was measured in response to control or grain digest treatments. Control CFS+Amylase (blue) vs CCS+Amylase (red) (top left). Oral bacteria grown in fine (blue) or coarse (red) grain digests from 2.5 min digestion (p=0.021) (top right), 5 min (p=0.025) (bottom left), 10 min (p=0.034) (bottom middle), and 20 min (p=0.023) (bottom right). Shaded regions represent 95% confidence intervals. Significant differences were identified using Student's t-test.

## Discussion

The findings of this chapter provide foundational mechanistic evidence that food particle size, as a proxy for degree of grain processing, exerts a direct and measurable influence on oral biofilm abundance, architecture, and growth kinetics. Two complementary lines of evidence support this conclusion: the *in vivo* dietary hardness experiment, in which animals fed a soft diet accumulated substantially greater biofilm burden than those fed an equivalent hard diet, and the *in vitro* grain digest model, in which fine-particle (i.e., processed) grain digests promoted more rapid bacterial growth and larger biofilm surface areas than coarse-particle digests. Together, these data suggest that grain processing compromises oral biofilm regulation through two mechanistically distinct but synergistic pathways: removal of the physical-mechanical resistance of grain texture,

and augmentation of the saccharide substrate available to early oral colonizers via enhanced amylolytic starch hydrolysis.

The *in vivo* finding that dietary hardness markedly reduces biofilm loading on murine dental surfaces is consistent with the established role of masticatory mechanical forces in limiting oral biofilm accumulation.[27,28,29] Dental surfaces are non-shedding hard tissues that, unlike the mucosal epithelium, cannot renew their surface layer to resist microbial colonization.[22,23] In the absence of adequate masticatory challenge, the dentogingival surface lacks the intermittent mechanical disruption that limits biofilm progression from early colonization to mature, structured communities. The stark contrast in biofilm abundance between hard and soft diet animals observed by SEM in this study, despite nutritional equivalence, implicates dietary texture as an independent determinant of biofilm accumulation, separable from the metabolic consequences of dietary composition. This is consistent with epidemiological observations that populations consuming predominantly soft, processed diets exhibit elevated rates of periodontal disease even when confounders including sugar intake are partially controlled.[13,14,15]

The *in vitro* grain digest experiments extend these observations by interrogating the metabolic pathway through which grain processing promotes biofilm dysbiosis. Fine-particle grain digests, reflecting the increased amylolytic surface area exposed following removal of the fibrous bran encapsulation,[7,9,30] yielded significantly greater bacterial growth kinetics across all digestion intervals tested. This dose-dependent enhancement of growth at each digestion time point argues against a simple confound of initial substrate concentration and instead supports the interpretation that grain processing fundamentally alters the kinetics and magnitude of saccharide release by salivary amylase. Salivary amylase hydrolyzes alpha-1,4 glycosidic linkages in starch to yield glucose, maltose, and maltodextrins, fermentable substrates utilized by early oral colonizers

including amylase-binding streptococcal species, which constitute a major component of supragingival plaque.[19,20,21] Enrichment of these saccharolytic pioneer species promotes acid production and pH depression at the plaque-tooth interface, creating selective conditions that favor the progressive enrichment of later-colonizing taxa with dysbiotic potential.[31,32]

The striking differences in biofilm architecture observed between coarse and fine grain conditions in the SEM images are particularly informative. Biofilm structure is not merely a consequence of microbial abundance but reflects the underlying community composition, species interactions, and extracellular matrix production, all of which collectively determine the metabolic and pathogenic potential of the biofilm community.[22,23,24] The observation that fine grain digests supported biofilms with nearly double the surface area of coarse grain counterparts suggests that the altered substrate environment does not simply increase total bacterial growth but actively reshapes biofilm architecture. This has important implications for periodontal pathogenesis: a more architecturally complex biofilm provides greater spatial opportunity for the integration of secondary and late-stage colonizers, including orange and red complex species, whose colonization is contingent upon prior establishment of a structurally mature early community.[23,33]

An important limitation of this preliminary work is the small sample size for the *in vivo* component (n=1 per group), which precludes statistical inference and must be interpreted as a qualitative, hypothesis-generating observation rather than a definitive empirical finding. The *in vitro* model, while providing internally replicated and statistically significant growth kinetic data, does not capture the full complexity of the *in vivo* oral environment, including salivary flow dynamics, host immune surveillance, the structural geometry of the dentogingival niche, and the polymicrobial inoculum diversity present in clinical periodontitis. Despite these limitations, the collective findings of this chapter were instrumental in defining the experimental logic and specific

research questions of the main in vivo study described in subsequent chapters. Three key conclusions from this preliminary work directly informed the design of the multi-diet periodontal model:

First, the *in vivo* dietary hardness experiment provided direct experimental confirmation that diet texture, independent of nutritional composition, is sufficient to regulate oral biofilm accumulation. This finding motivated the WG vs. PG dietary comparison in the main study: by testing grain diets that were structurally and compositionally matched except for the disruption of grain architecture and removal of the fibrous bran layer, the main study was designed to interrogate whether the biofilm-regulatory consequences of dietary texture observed here translate into measurable differences in periodontal health outcomes including alveolar bone morphology, gingival inflammatory cytokine profiles, and oral microbial community composition at the genus level.

Second, the *in vitro* growth kinetic data established that the saccharide substrate liberated by salivary amylase from fine-particle (i.e., processed) grain digests is sufficient to significantly augment the growth potential of the oral microbiota across a range of digestion time intervals. This directly raised the question of whether increased fermentable saccharide availability driving dysbiotic enrichment operates independently from the textural pathway, or whether both are required for the full periodontal phenotype observed in populations consuming processed grain diets.[1,2,3] To disentangle these pathways, the main study incorporated the FB vs. PB dietary comparison, in which fiber was supplemented in the absence of grain texture. If the metabolic pathway were sufficient, fiber bar supplementation would be expected to exert protective effects on microbiome composition and periodontal outcomes comparable to whole grain consumption.

Third, the *in vitro* biofilm architecture data, demonstrating that grain particle size shapes not merely biofilm quantity but biofilm structural organization, raised the question of whether diet-induced differences in biofilm architecture could be captured at the community composition level using 16S rRNA gene sequencing. The oral microbiome analysis in the main study was specifically designed to assess whether whole grain vs. processed grain diets give rise to compositionally distinct microbial communities, and whether those compositional differences correspond to differential representation of dysbiosis-associated taxa including *Enterococcus*, *Staphylococcus*, *Fusobacterium*, and members of the proteolytic anaerobic genera implicated in periodontal disease pathogenesis.[33,34]

Taken together, the preliminary data presented in this chapter established proof-of-concept for both the texture-mediated and substrate-mediated pathways through which grain processing may promote oral biofilm dysbiosis and provided the mechanistic rationale for an in vivo multi-diet experimental design capable of distinguishing their relative contributions to periodontal host-microbe homeostasis. The findings positioned dietary texture as a biologically plausible and experimentally testable modifier of periodontal outcomes, distinct from, and potentially more impactful than, fiber supplementation alone in the absence of food texture.

## Methods

### *Soft vs. hard diet regimen.*

To gain insight as to how food hardness impacts oral biofilm abundance in the presence of masticatory forces, 4-week old Sprague Dawley rats (Charles River Laboratories, Inc., Wilmington, MA, USA) were fed one of two nutritionally equivalent foods: hard pellet food (Hard Food = HF; n=1) or soft powdered chow (Soft Food = SF; n=1) (PicoLab 5058, LabDiet, Deans Animal Feeds, Redwood City, CA, USA) for 6 weeks; foods differed only in hardness (softer food was a powdered version of the pelleted chow).

### *Scanning electron microscopy.*

Right hemimandibles (n=1) were dissected and carefully attached to titanium stubs using carbon tape (occlusal surfaces up). Images were then acquired using SIGMA VP500 (Carl Zeiss Microscopy, Pleasanton, CA) at 1 keV voltage.

### *Human saliva collection and informed consent.*

Human saliva collection was approved by the University of California San Francisco Institutional Review Board (IRB #17-21912, Reference #186994). Ten healthy volunteers verbally consented to donate saliva for this study. No information from the volunteers was collected prior to or at the time of saliva donation. Prior to collection, volunteers were informed not to eat, drink, and/or smoke for at least 30 minutes prior to donation. Approximately 10–15 mL of saliva was obtained from each volunteer. All collected saliva was pooled, centrifuged at 10,000 × RPM for 30 min, and separated into cell-containing saliva (CCS) and cell-free saliva (CFS). CCS was obtained by adding glycerol (50% v/v) to the precipitate of the centrifuged pooled saliva, stored at −80°C, and was used as the biofilm inoculum. CFS was obtained by collecting the supernatant of

the centrifuged pooled saliva, diluted with sterile phosphate-buffered saline (PBS) (1:4 v/v), stored at −80°C, and used as the medium.

***Grain preparation.***

Wheat berries (Soft White Wheat Berries, Palouse Brand, Palouse, WA) were autoclaved and milled into either coarse or fine particles (WonderMill Grain Grinder, Pleasant Hill Grain, Nebraska, USA): coarse particles were created using the "Coarse" setting, and fine particles were created using the "Pastry" setting on the grain grinder with a 1250-watt motor.

***Grain digestion.***

Twenty mg of coarse or fine grain particles were placed in a sterile 1.5 mL Eppendorf tube, briefly vortexed to settle particles, and suspended in 1 mL of CFS. Samples were vortexed for 5 s and subjected to digestion by salivary amylase present in saliva for either 2.5-, 5-, 10-, or 20-min digestion intervals at 34.5°C. Following this, grain particles were centrifuged for 10 min at 14,000 × rpm at 4°C. 800 µL supernatant (coarse or fine grain digest) was collected in a sterile 1.5 mL Eppendorf tube. Tubes were wrapped in parafilm to prevent contamination. Samples were then heat-shocked in a water bath for 5 min at 90°C to halt amylase activity. Samples were then cooled on ice for 10 min.

***Biofilm growth.***

Sterilized round glass coverslips were placed in 24-well plates and served as the surface for biofilm growth. Human saliva-derived oral biofilms were grown by adding 15 µL of CCS to 485 µL of coarse or fine grain digest per well in 24-well plates (n=4). Biofilms were incubated under aerobic conditions for 24 h at 37°C in a humidified Thermo-Fisher Forma Series II Incubator.

***Scanning electron microscopy (biofilms).***

After 24 hours of growth, spent media was removed and biofilms were gently rinsed with PBS three times. 300 µL of 2.5% glutaraldehyde in 0.1 M phosphate buffer was then added to wells for 20 min. Fixative was removed and biofilms were stored in PBS overnight at 4°C. Biofilms were washed twice with PBS and subjected to dehydration using a graded ethanol series (10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% EtOH) at 5 min intervals. Biofilms were then coated with gold/palladium (Au/Pd) sputter coating at 1 nm thickness (Leica EM ACE600). Glass coverslips were carefully removed from wells and attached to titanium stubs using carbon tape (biofilm side up). Images were acquired using SIGMA VP500 (Carl Zeiss Microscopy, Pleasanton, CA) at 1 keV voltage.

***Growth curve.***

Twenty-four-hour spectrophotometer readings were captured for oral bacteria grown in either coarse or fine grain digests. 485 µL of grain digest was warmed to 37°C. 15 µL of CCS was then added, and the mixture was gently resuspended three times. 100 µL was plated into a 96-well plate and grown for 12 hours at 660 nm with 15 min interval readings using a microplate reader (SpectraMax M2, Molecular Devices, Sunnyvale, CA, USA). Microbial growth was analyzed by quantifying and comparing area under the curve (n=4).

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## Chapter 3: Diet texture, not fiber content, modulates host markers related to periodontitis in BALB/cByJ mice

### Introduction

Oral biofilms become more pathologic as maturation progresses, as a higher representation of pathogens is facilitated within the system.[1,2] Abrasive diets have been shown to reduce biofilm accumulation, which exists as a primary etiology in gingival inflammation and ultimately periodontitis.[3,4,5] Dietary carbohydrates, particularly refined and processed variants, represent a significant environmental determinant in the initiation and progression of periodontal disease. Fermentable carbohydrates serve as primary substrates for oral biofilm metabolism, facilitating the proliferation of acidogenic and saccharolytic species such as *Streptococcus mutans*, *Lactobacillus* spp., and *Fusobacterium nucleatum*.[6,7] The resultant metabolic byproducts in tandem with excessive biofilm loading directly compromise gingival epithelial integrity and promote a pro-inflammatory tissue environment conducive to periodontal breakdown.[8] As such, refined carbohydrate consumption promotes dysbiosis by increasing abundance of primary and secondary colonizers, instating inflammation, and ultimately enriching the subgingival microbiome with keystone periodontal pathogens, including *Porphyromonas gingivalis*, *Tannerella forsythia*, and *Treponema denticola*, whose virulence factors actively subvert innate immune surveillance.[9,10]

Moreover, a unique protective inflammatory response has been observed in gingival tissues in response to dietary hardness,[11,12] characterized by microbiota-independent, IL-6-dependent production of IL-17 by Th17 cells and γδ-T cells. This physiological IL-17 response is fundamentally distinct in both its cellular origin and functional consequence from the pathological

IL-17 signaling observed in periodontitis. Unlike dysbiosis-dependent, IL-23-driven expansion of Th17 cells that characterizes established periodontal disease,[13,14] the mechanically induced IL-17 response is elicited in the absence of microbial dysbiosis, is not contingent on IL-23 signaling, and does not drive osteoclastogenesis.[11,15] Rather, it operates as a homeostatic response, in which mechanosensory stimulation of the gingival epithelium by abrasive dietary substrates activates resident Th17 and γδ-T cells through IL-6-mediated mechanisms, independent of adaptive immune priming.[11,16] The IL-6 dependency of this response further distinguishes it from pathological Th17 activation in that IL-6, in the absence of IL-23, is insufficient to drive the Th17 stabilization and osteoclastogenesis characteristic of periodontal disease.[11,13]

The stimulation and outcomes of the physiological IL-17 response are distinct to the oral cavity. Enhanced gingival barrier function, demonstrated by increased representation of defensins, neutrophil chemo-attractants, and neutrophils in healthy gingival tissues, has also been observed in response to increased dietary hardness.[17] In this context, IL-17 acts coordinately with epithelial-derived alarmins to upregulate the expression of β-defensins, S100 proteins, and CXCL chemokines, reinforcing barrier competency and priming a state of immunological readiness without initiating destructive inflammation.[18,19] Thus, the gingival immune architecture, shaped by dietary hardness, represents barrier reinforcement in place of pathological inflammation. This physiological response is lost with processed food consumption, in that abrasive substrates provided by maintenance of intact fiber structure are replaced with soft, fermentable carbohydrates that lack mechanical challenge.

In periodontitis, degradation of epithelial barrier proteins such as E-cadherin and beta-catenin occurs, resulting in reduced gingival barrier integrity and function.[20] Local expansion of pathological IL-17+/CD4+ T helper (Th17) cells also occurs,[13,14] which is dependent on microbial

dysbiosis, IL-6, and IL-23. Moreover, periodontal inflammation is characterized by high neutrophil counts.[21] High Th17 cell and neutrophil populations are maintained in inflamed gingival tissues via reciprocal reinforcement, mediated primarily via the action of inflammatory cytokines IL-6, IL-17, IL-23, tumor necrosis factor-alpha (TNF-α), interferon-gamma (IFN-γ), granulocyte-colony stimulating-factor (G-CSF), as well as CXCL8, CCL2, and CCL20 chemokines.[21,22] Such inflammatory mediators maintain the periodontal tissues in an inflammatory state, further amplifying inflammatory responses and driving microbial dysbiosis. It is this relentless loop of dysbiosis and inflammation that initiates and progresses periodontitis, ultimately leading to destruction of soft and hard tissues comprising the periodontal complex. In addition to microbial dysbiosis, the relationship between dietary carbohydrate quality and reduced induction of mechanically stimulated gingival barrier function underscores a potential mechanistic link between processed food consumption and periodontal susceptibility.[11,13]

Here, the effects of diet texture and dietary fiber on periodontal disease-associated immune markers were investigated to better understand if food processing, and more specifically grain refinement, alters inflammatory processes observed in periodontitis. Five diet groups were tested, including: Standard Diet (SD), Whole Grain (WG) vs Processed Grain (PG), and Fiber Bar (FB) vs Placebo Bar (PB). The WG vs PG diets were chosen to investigate if grain processing alters gingival inflammatory responses and periodontal bone loss via disruption of grain integrity and resultant loss of fiber content and diet texture. Fiber Bar (FB) and Placebo Bar (PB) groups were added to further delineate if the presence or absence of fiber, rather than the structural and nutritional changes evoked by dietary texture, elicit changes on the same inflammatory markers of periodontitis. To control for this, the FB and PB diets only differed nutritionally in that the FB included a proprietary fiber blend whereas PB did not. All diet regimens were tested both in the

presence and absence of inoculation with periodontal pathogens, experimentally executed via an oral lavage model of periodontitis.[23]

## Results

### *Diet is the dominant variable, not inoculation status, on global cytokine profiles between sham and inoculated animals.*

Left hemimandibles were collected for each group (n=4; 2 males, 2 females) and gingival tissues were used to assess potential differences in levels of cytokines that are well-recognized in the inflammatory etiology of periodontitis (TNF-α, IL-1β, IL-6, IL-23, IL-17A, and IL-10).[24,25] Global PERMANOVA comparisons were performed on cytokine datasets across inoculated groups, diet groups, and combined diet × inoculated groups. Across all sham samples, diet explained significant variation in cytokine profiles ($R^2$ = 0.22, P = 0.001) (**Supplemental Fig. 3.1a**). However, inoculated status alone did not significantly explain variation in cytokine profiles ($R^2$=0.01, p=0.98) (**Supplemental Fig. 3.1b**). When all diet × inoculated groups were included, the strongest separation was observed ($R^2$ = 0.35, P = 0.008) (**Supplemental Fig. 3.1c**).

Significant differences were not observed for overall inflammatory cytokine profiles between sham vs inoculated groups within the same diet regimen (**Supplemental Fig. 3.2**): SD_sham vs SD_inoc (PERMANOVA; $p$>0.05, $R^2$=0.19; **Supplemental Fig. 3.2a**), WG_sham vs WG_inoc (PERMANOVA; $p$>0.05, $R^2$=0.15; **Supplemental Fig. 3.2b**), PG_sham vs PG_inoc (PERMANOVA; $p$>0.05, $R^2$=0.14; **Supplemental Fig. 3.2c**), FB_sham vs FB_inoc (PERMANOVA; $p$>0.05, $R^2$=0.11; **Supplemental Fig. 3.2d**), and PB_sham vs PB_inoc groups (PERMANOVA; $p$>0.05, $R^2$=0.18; **Supplemental Fig. 3.2e**). A lack of evidence supporting significantly greater inflammatory response among inoculated groups suggested that differences

observed between dietary groups may have been driven by dietary regimen as opposed to assigned inoculated status. Thus, pairwise ordinations between diet groups within inoculated strata were performed to investigate the role of diet on inflammatory cytokine profiles (e.g., WG vs PG, FB vs PB, and WG vs FB).

No significant differences were observed among IL-17A cytokine profiles between sham vs inoculated groups within the same diet regimen (**Supplemental Fig. 3.3**): SD_sham vs SD_inoc (Wilcoxon rank sum test; $p>0.05$; **Supplemental Fig. 3.3a**), WG_sham vs WG_inoc (Wilcoxon rank sum test; $p>0.05$; **Supplemental Fig. 3.3b**), PG_sham vs PG_inoc (Wilcoxon rank sum test; $p>0.05$; **Supplemental Fig. 3.3c**), FB_sham vs FB_inoc (Wilcoxon rank sum test; $p>0.05$; **Supplemental Fig. 3.3d**), nor PB_sham vs PB_inoc groups (Wilcoxon rank sum test; $p>0.05$; **Supplemental Fig. 3.3e**). These findings further implicate that diet regimen, not inoculation status, is responsible for significant differences observed between groups.

***Diet texture, not fiber content, modulates the gingival immune cytokine environment.***

Each diet was designed with an intrinsic matched control, allowing isolation of specific dietary variables of interest while minimizing confounding nutritional differences. Specifically, the WG and PG diets each consisted of 50% SD and 50% grain component by weight and were directly compared to test the effect of whole versus processed grain inclusion. Similarly, the FB and PB diets consisted of 20% bar and 80% SD by weight and were compared to assess the effect of blended fiber supplementation versus a matched placebo. Comparison of WG vs FB was used to interrogate the effect of diet texture attributable to fiber content versus blended fiber supplementation.

Global gingival cytokine profiles were assessed to evaluate the effects of dietary regimen on inflammatory signaling associated with periodontal disease (**Fig. 3.1**). Among sham animals,

no significant differences in overall cytokine profiles were observed between WG_sham and PG_sham groups (PERMANOVA, $p > 0.05$, $R^2 = 0.19$; **Fig. 3.1a**). Similarly, comparison of inoculated animals revealed no significant differences between WG_inoc and PG_inoc groups (PERMANOVA, $p > 0.05$, $R^2 = 0.25$; **Fig. 3.1b**). As significant differences were not observed between sham and inoculated groups within each diet regimen, sham and inoculated groups were combined to assess the effect of diet on cytokine profiles irrespective of inoculated status. When pooled, a significant difference in overall cytokine profiles was detected between WG_All and PG_All groups (PERMANOVA, $p = 0.03$, $R^2 = 0.15$; **Fig. 3.1c**). To identify individual cytokines contributing to this difference, univariate analyses were performed. IL-17A levels were significantly higher in PG_All compared to WG_All animals (Wilcoxon rank-sum test, $p = 0.043$; **Fig. 3.2a**). IL-17A is a cytokine with a well-established role in inflammatory alveolar bone loss in periodontitis[13,14,26].

In contrast, no significant differences in overall cytokine profiles were observed between fiber bar and placebo bar diets among sham (FB_sham vs PB_sham; PERMANOVA, $p > 0.05$, $R^2 = 0.17$; **Fig. 3.1d**), inoculated (FB_inoc vs PB_inoc; PERMANOVA, $p > 0.05$, $R^2 = 0.23$; **Fig. 3.1e**), or pooled groups (FB_All vs PB_All; PERMANOVA, $p > 0.05$, $R^2 = 0.09$; **Fig. 3.1f**). To further evaluate whether differences between whole grain and fiber bar diets were attributable to fiber content or dietary form, WG and FB groups were compared directly. No significant differences in overall cytokine profiles were detected between WG_sham and FB_sham groups (PERMANOVA, $p > 0.05$, $R^2 = 0.21$; **Fig. 3.1g**) or between WG_inoc and FB_inoc groups (PERMANOVA, $p > 0.05$, $R^2 = 0.31$; **Fig. 3.1h**). In contrast, when inoculated status was pooled, a significant difference in overall cytokine profiles was observed between WG_All and FB_All groups (PERMANOVA, $p = 0.017$, $R^2 = 0.19$; **Fig. 3.1i**). Subsequent univariate analysis identified

IL-17A as a key contributor to this difference, with significantly higher IL-17A levels detected in FB_All compared to WG_All animals (Wilcoxon rank-sum test, $p = 0.0095$; **Fig. 3.2b**).

***Dietary regimen, not inoculation status, is the dominant variable in mediating alveolar bone loss.***

Micro-CT scans of right hemimaxilla were used to assess alveolar bone loss (ABL) within each group (n=4; 2 males, 2 females). Three technical replicates were used per sample. For each slice, total ABL was determined as the sum of distances from the alveolar bone crest to the cemento-enamel junction (ABC-CEJ) at four sites (M1 crest, M1 furcation, M1/M2 contact, and M2 furcation). The mean of the three technical replicates was calculated and assigned as the total bone loss per sample. Total bone loss across samples was compared between sham and inoculated groups (**Supplemental Fig. 3.4**). No significant differences were observed for SD_sham vs SD_inoc (Wilcoxon rank sum test; $p>0.05$; **Supplemental Fig. 3.4a**), WG_sham vs WG_inoc ($p>0.05$; **Supplemental Fig. 3.4b**), PG_sham vs PG_inoc ($p>0.05$; **Supplemental Fig. 3.4c**), FB_sham vs FB_inoc ($p>0.05$; **Supplemental Fig. 3.4d**), or PB_sham vs PB_inoc ($p>0.05$; **Supplemental Fig. 3.4e**). These findings implicate that polymicrobial inoculation did not elicit significant differences in ABL between sham and inoculated groups subjected to the same diet regimen.

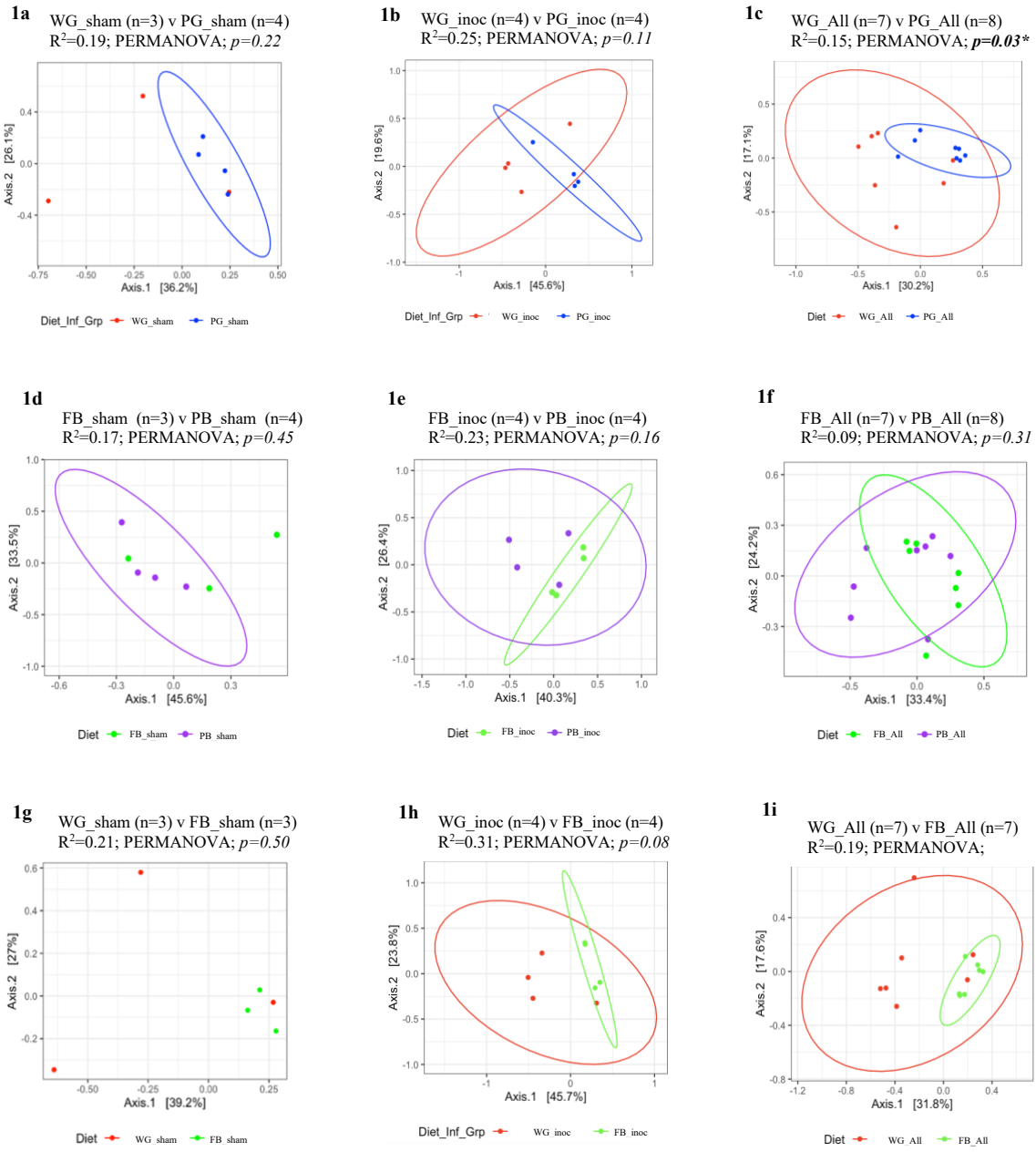


**Figure 3.1. Overall cytokine levels.** Principal coordinate analysis (PCoA) plots based on Canberra distance matrices depict global gingival cytokine expression profiles across dietary regimens and inoculated states. Each plot displays the first two principal coordinate axes with 95% confidence ellipses surrounding group centroids. **1a.** WG_sham vs PG_sham ($R^2$ = 0.19, $p$ = 0.22): no significant difference in overall cytokine profiles. **1b.** WG_inoc vs PG_inoc ($R^2$ = 0.25, $p$ = 0.11): no significant difference between inoculated groups. **1c.** WG_All vs PG_All ($R^2$ = 0.15, $p$ = 0.03): cytokine profiles differed significantly between diets when sham and inoculated status were pooled. **Figure legend continued next page.**

**Figure legend continued from previous page. 1d.** FB_sham vs PB_sham ($R^2 = 0.17$, $p = 0.45$): no significant difference. 1**e.** FB_inoc vs PB_inoc ($R^2 = 0.23$, $p = 0.16$): no significant difference. **1f.** FB_All vs PB_All ($R^2 = 0.09$, $p = 0.31$): no significant difference. **1g.** WG_sham vs FB_sham ($R^2 = 0.21$, $p = 0.50$): no significant difference. **1h.** WG_inoc vs FB_inoc ($R^2 = 0.31$, $p = 0.08$): no significant difference. **1i.** WG_All vs FB_All ($R^2 = 0.19$, $p = 0.017$): cytokine profiles differed significantly between diets when inoculated status was pooled. Statistical differences were assessed using PERMANOVA. Asterisks indicate $p < 0.05$.

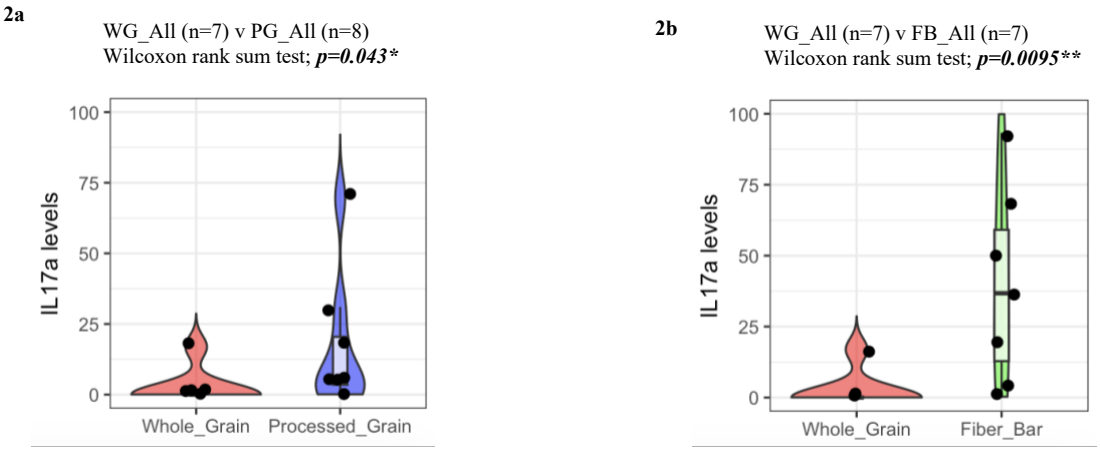


**Figure 3.2. IL-17 profiling.** Violin plots depict IL-17A protein levels in gingival tissues of mice fed different diets. **2a.** WG_All (n=7) vs PG_All (n=8): Whole grain diet was associated with significantly lower IL-17A expression compared to processed grain (p=0.043*). **2b.** WG_All (n=7) vs FB_All (n=7): IL-17A levels were significantly reduced in the whole grain group compared to fiber bar-fed mice (p=0.0095**). Statistical comparisons were performed using the Wilcoxon rank-sum test. Asterisks denote significance: *p < 0.05, **p < 0.01.

***Diet texture, not fiber content, is the dominant variable mediating alveolar bone loss.***

Alveolar bone loss (ABL) was quantified to assess the relative effects of dietary regimen and inoculated status on periodontal bone outcomes (**Fig. 3.3**). Mechanical testing confirmed a significant difference in food hardness between WG and PG diets (n=3; two-tailed *t*-test, $p = 0.011$), indicating that greater force was required to produce the same displacement in the WG diet compared to the PG diet (**Supplemental Fig. 3.5**). Among sham animals, WG_sham mice exhibited significantly less ABL compared to PG_sham mice (Wilcoxon rank-sum test, $p = 0.029$; **Fig. 3.3a**). In contrast, no significant difference in ABL was observed between WG_inoc and PG_inoc groups ($p > 0.05$; **Fig. 3.3b**). When inoculated status was pooled, PG_All mice demonstrated significantly greater ABL compared to WG_All mice (Wilcoxon rank-sum test, $p = 0.045$; **Fig. 3.3c**). No significant differences in ABL were detected between FB and PB diets among sham ($p > 0.05$; **Fig. 3.3d**), inoculated ($p > 0.05$; **Fig. 3.3e**), or pooled groups ($p > 0.05$; **Fig. 3.3f**). Exploratory comparisons of WG vs FB groups revealed a trend toward greater ABL in FB_sham compared to WG_sham mice ($p = 0.057$; **Fig. 3.3g**) and in FB_inoc compared to WG_inoc mice ($p = 0.057$; **Fig. 3.3h**). When inoculated status was pooled, FB_All mice exhibited significantly greater ABL than WG_All mice (Wilcoxon rank-sum test, $p = 0.00056$; **Fig. 3.3i**).

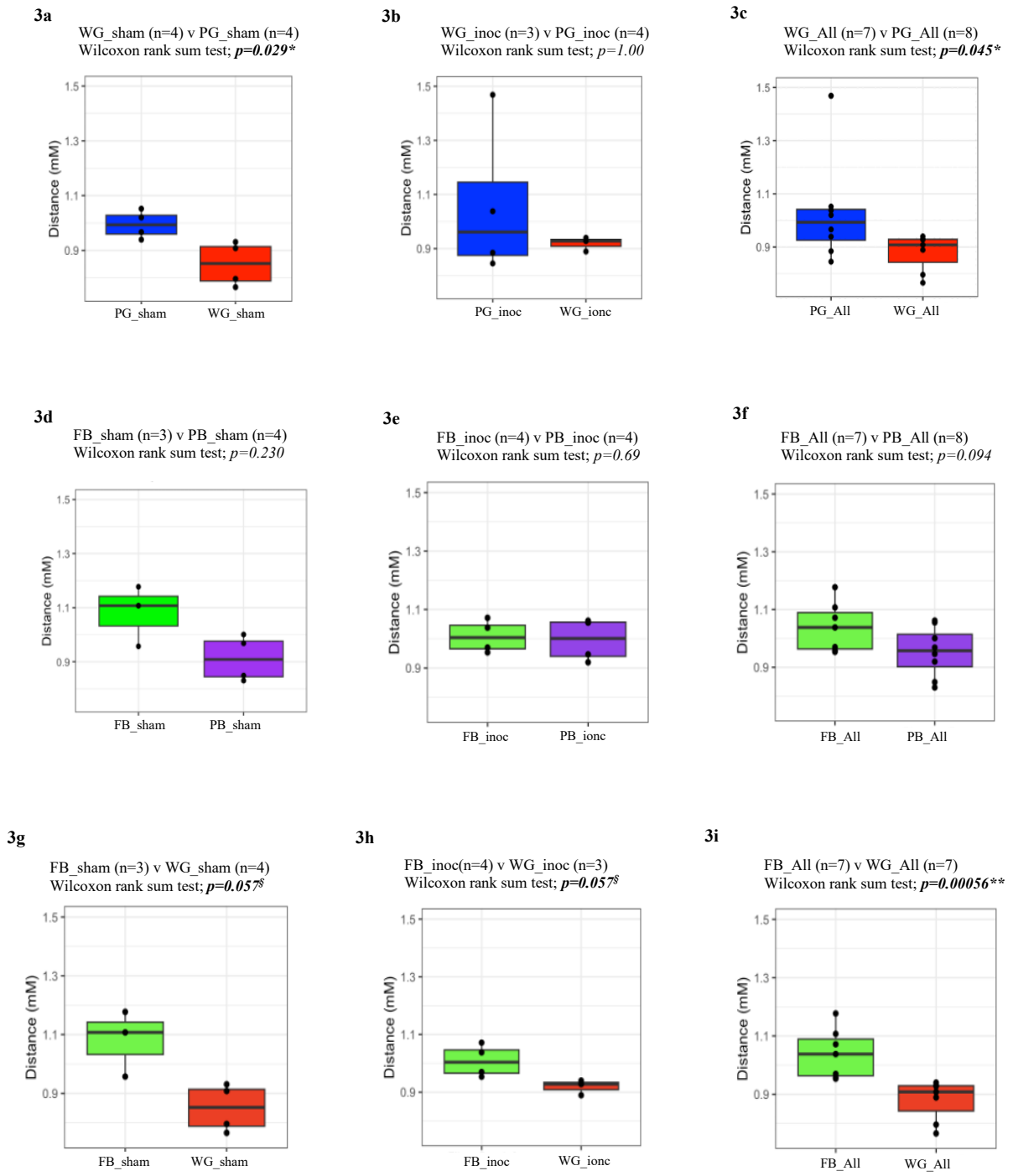


**Figure 3.3. Alveolar bone loss.** Box plots show alveolar bone loss (ABL) in millimeters across dietary and inoculated conditions. **3a.** PG_sham vs WG_sham (p=0.029*), **3b.** PG_inoc vs WG_inoc (p=1.00), **3c.** PG_All vs WG_All (p=0.045*), **3d.** PB_sham vs FB_sham (p=0.230), **3e.** PB_inoc vs FB_inoc (p=0.69), **3f.** PB_All vs FB_All (p=0.094), **3g.** FB_sham vs WG_sham (p=0.057), **3h.** FB_inoc vs WG_inoc (p=0.057), **3i.** FB_All vs WG_All (p=0.00056**). Statistical differences between groups were measured using the Wilcoxon rank-sum test. Asterisks denote significance: *p < 0.05, **p < 0.01. § Denotes trending significance.

***IL-17A levels positively associate with alveolar bone loss among diet groups.***

Supporting a mechanistic link between inflammation and alveolar bone loss, a Pearson correlation analysis revealed a statistically significant positive relationship between IL-17A levels and total bone loss across all dietary groups ($R = 0.47$, $p = 0.0096$; **Fig. 3.4**). Whole Grain subjects consistently occupied the lower end of both axes, reinforcing the interpretation that reduced IL-17A expression in this group corresponded to attenuated bone loss. Animals consuming a Whole Grain diet exhibited notably lower total bone loss compared to other dietary groups, clustering tightly at the lower end of the bone loss axis (approximately 0.75–0.95) with correspondingly minimal IL-17A levels (near 0–18 pg/mL). This pattern suggests that Whole Grain consumption was associated with both reduced skeletal deterioration and attenuated pro-inflammatory cytokine expression.

In contrast, Fiber Bar and Processed Grain animals demonstrated greater variability in bone loss, with several individuals extending toward higher values (up to ~1.1 and ~1.47, respectively), and in some cases markedly elevated IL-17A levels. Placebo Bar subjects similarly clustered at low bone loss values but showed slightly more spread than the Whole Grain group. The comparatively low positioning of Whole Grain subjects along both axes is consistent with a protective dietary effect, whereby whole grain consumption may mitigate IL-17A-driven inflammatory signaling and its downstream consequences on bone integrity.

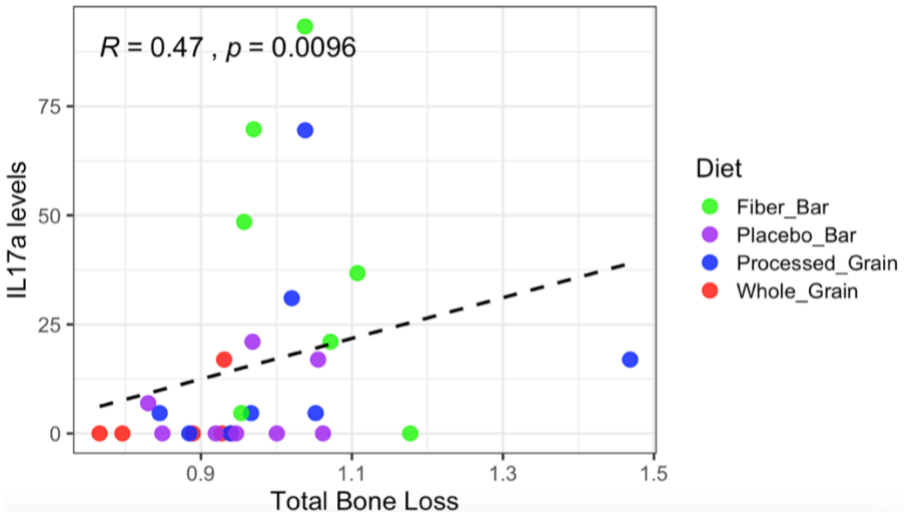


**Figure 3.4. Correlation between IL-17A levels and total bone loss across dietary groups.** Scatter plot depicts the relationship between total bone loss (mm, x-axis) and gingival IL-17A protein levels (pg/mL, y-axis) across all animals, colored by dietary group: Whole Grain (red), Processed Grain (blue), Fiber Bar (green), and Placebo Bar (purple). The dashed line represents a linear regression fit. A statistically significant positive correlation was observed across all groups (Pearson R = 0.47, p = 0.0096), indicating that greater bone loss was associated with higher IL-17A levels. Whole Grain animals clustered at the lower end of both axes, consistent with reduced IL-17A levels and bone loss in this dietary group.

## Discussion

The presented findings suggest that dietary texture, and not fiber content, is the dominant dietary modifier of the gingival immune cytokine environment and alveolar bone morphology in a murine model of experimental inoculation with periodontal pathogens. Across multiple dietary comparisons, significant differences in both global cytokine profiles and ABL were consistently observed between the WG and PG diet groups, which differed in both texture and intrinsic fiber content, and between WG and FB diet groups, which differed primarily in texture while delivering comparable levels of dietary fiber through different forms (intact grain versus supplemented blend). In contrast, no significant differences in cytokine profiles or ABL were detected between the FB and PB groups, which differed only in fiber supplementation in the absence of textural

distinction. Taken together, the pattern of results across all three diet comparisons converges on a single conclusion: the structural and mechanical properties of food, not its fiber content in isolation, are the primary dietary determinants of gingival immune homeostasis and alveolar bone preservation.[11,12]

A particularly notable finding of this study is that diet, rather than polymicrobial inoculation with periodontal pathogens, explained the preponderance of variation in both cytokine profiles and alveolar bone outcomes. Global PERMANOVA analysis demonstrated that diet accounted for significantly more variance in gingival cytokine composition than inoculation status, and pairwise comparisons within each diet group revealed no significant differences between sham and inoculated animals. This result underscores the importance of dietary context in shaping the baseline inflammatory set point of gingival tissues, independently of direct microbial challenge.

Among the cytokines profiled, IL-17A emerged as the key mediator discriminating dietary groups. Significantly elevated IL-17A was detected in PG_All compared to WG_All animals, and in FB_All compared to WG_All animals, with both findings mirroring the corresponding differences in alveolar bone loss. The role of IL-17A in driving periodontal bone destruction is well established: via RANKL upregulation in periodontal ligament cells, osteoblasts, and stromal fibroblasts, IL-17A promotes osteoclastogenesis and net alveolar bone resorption through both direct and indirect mechanisms.13,14,25,29 IL-17A also amplifies gingival inflammation through NF-κB and STAT3 pathway activation, driving expression of CXCL8 and CCL20 that sustain neutrophil recruitment and perpetuate the inflammatory loop characteristic of progressing periodontitis.[21,30] The co-occurrence of elevated IL-17A and greater ABL in the PG and FB groups therefore suggests that the loss of dietary texture, whether via grain processing or via provision of

fiber in a supplemented, blended form, creates conditions permissive for pathological IL-17A-driven osteoclastogenesis.

The positive correlation between gingival IL-17A levels and total bone loss observed across all dietary groups provides further mechanistic support for the interpretation that diet-driven differences in IL-17A expression translate directly into differential alveolar bone outcomes. Whole Grain animals consistently occupied the lower end of both axes, exhibiting simultaneously the lowest IL-17A levels and the least total bone loss, while Fiber Bar and Processed Grain animals demonstrated greater dispersion along both dimensions. This distributional pattern reinforced the group-level cytokine and ABL findings and suggests that IL-17A is not merely a correlate of disease severity in this model, but a plausible mechanistic intermediary through which dietary texture exerts its protective effects on alveolar bone.[13,25,29,37] The direction and magnitude of this association are consistent with prior reports linking elevated gingival IL-17A to progressive alveolar bone resorption in both murine experimental periodontitis models and human periodontal disease, further supporting the biological relevance of the diet-driven IL-17A differences observed here.[21,25,29,38]

These findings take on interpretive significance considering the established literature on the dual roles of IL-17 at the oral mucosal barrier. Seminal work by Dutzan and colleagues demonstrated that physiological barrier damage from mastication induces IL-6 production from gingival epithelial cells, which in turn drives the accumulation of homeostatic IL-17-producing Th17 cells at the gingiva through a mechanism that is commensal-independent and IL-23-independent.11 This masticatory-mechanosensory IL-17 response is fundamentally distinct from the pathological dysbiosis-driven Th17 response in that it does not depend on IL-23, does not promote osteoclastogenesis, and instead reinforces gingival barrier function through upregulation

of β-defensins, S100 alarmin proteins, and CXCL chemokines that prime epithelial antimicrobial defense.[11,17,18] The present data are consistent with a model in which the WG diet, by providing ongoing masticatory mechanical challenge, sustains this homeostatic IL-17 signaling axis and its attendant barrier-reinforcing consequences, thus resulting in lower steady-state gingival IL-17A and decreased alveolar bone loss. Conversely, the PG and FB diets, lacking equivalent mechanical challenge, may fail to adequately sustain the physiological mechanosensory input required to maintain this homeostatic immune tone, predisposing the gingival immune environment toward pathological IL-17A accumulation when dysbiosis-permissive conditions develop.

The present findings have direct implications for understanding how dietary texture interfaces with established pathways of gingival immune barrier function. The gingival barrier is a uniquely challenging epithelial niche; unlike keratinized mucosal epithelia, the junctional epithelium at the dentogingival junction is thin, highly permeable, and anchored only weakly to the tooth surface by hemidesmosomes, rendering it constitutively susceptible to both microbial and mechanical perturbation.[31,32] Resident γδ-T cells of the junctional epithelium, the dominant IL-17-producing lymphocyte population in the steady-state gingiva, are enriched at this vulnerable interface and have been shown to perform critical barrier surveillance and tissue repair functions.[15,16] This dietary hardness-dependent expansion of gingival γδ-T cells provides a mechanistic cellular basis for the IL-17 and Areg co-production that supports barrier integrity, and its apparent dependence on masticatory mechanical stimulation is directly relevant to the IL-17A differences observed across dietary groups in the present study.

Taken together, a mechanosensory-immune circuit emerges from the available evidence in which dietary texture generates mechanical shear forces at the dentogingival interface during mastication that stimulate gingival epithelial cells and trigger IL-6 release, epithelial IL-6 signals

to resident Th17 cells and γδ-T cells in an IL-23-independent manner, inducing physiological IL-17 production that reinforces barrier function and limits pathogen penetration.[11,15,17,18] The loss of dietary texture, as occurs with grain refinement, interrupts this circuit at its first step, depriving gingival epithelial cells of the mechanical stimulus required to sustain IL-6-driven homeostatic IL-17 signaling. In the absence of this homeostatic signal, the gingival barrier may be more vulnerable to dysbiosis-driven pathological Th17 expansion and the consequent IL-17A-mediated osteoclastogenesis and alveolar bone loss observed in the PG and FB groups.[13,14,25]

Several limitations of this study warrant consideration. First, the relatively small sample sizes per group limit statistical power. The exploratory nature of WG vs FB comparisons, several of which achieved statistical significance only when sham and inoculated groups were pooled, should be interpreted with caution. Replication in larger cohorts will be required to confirm the directionality and magnitude of these effects. Second, the use of a cytokine multiplex assay to profile only six cytokines (TNF-α, IL-1β, IL-6, IL-23, IL-17A, and IL-10) in tissue lysates provides a limited immunological window. The mechanosensory-barrier circuit described above implicates additional mediators (including amphiregulin, β-defensins, S100 proteins, CXCL chemokines, and RANKL/OPG) whose differential expression across dietary groups cannot be assessed from the present data. Future studies incorporating broader transcriptomic or proteomic profiling of gingival tissue would provide a more complete characterization of how dietary texture modulates the gingival immune landscape.[11,15,17]

The oral lavage model of periodontitis employed here, while reproducible and well-characterized,[22] failed to produce significant differences between sham and inoculated groups across nearly all dietary conditions. This suggests that the inoculum did not consistently establish a dysbiotic state sufficient to override diet-driven immune differences in this model. This may

reflect limitations of the timeline chosen for lavage inoculation approach, the immune competence of the BALB/cByJ strain, or the dominance of diet as an ecological determinant, all of which deserve further mechanistic dissection.

Finally, the dietary comparisons in this study cannot fully exclude that differences in macronutrient content between WG and FB diets, and minor nutritional components, including micronutrients, antioxidants, or non-nutritive bioactive compounds, between WG and PG diets, contributed to the observed differences in cytokine profiles and ABL. Future studies incorporating more precisely defined synthetic diets would further strengthen the attribution of these effects to dietary texture and mechanical properties.

## Methods

### *Study design.*

A power analysis was performed using analysis software (University of British Columbia: https://www.stat.ubc.ca/~rollin/stats/ssize/n2.html) and based on data from our previous study (Gao et al. 2020), relative to the mean and SD for alveolar bone loss between groups. All protocols were approved by the Institutional Animal Care and Use Committee of the University of California, San Francisco (UCSF; AN187219-01). 8-week-old BALB cByJ mice (40 males, 40 females) were purchased from Jackson Laboratories. Mice were housed under microisolator conditions (n=4 per isolator, single sex per isolator), provided water and food *ad libitum*, and were maintained at 25°C with alternating 12-hour light-dark cycles.

Mice were assigned to dietary groups at Week 2 and remained on diets for four weeks to establish an oral microbiota reflective of each diet regimen. At Week 6, mice within each dietary regimen were split into either sham-inoculated or inoculated subgroups (n=8; 4 males, 4 females). Among the inoculated subgroup, mice received a polymicrobial inoculum of periodontal pathogens applied topically to the gingiva 4×/week for four weeks. The sham group alternatively received a sham inoculum (2% CMC) 4×/week for four weeks (**Supplemental Fig. 3.6**).

### *Diet preparation.*

PicoLab® Rodent Diet 20, 5053 pellets were crushed until visibly homogenized and administered as the Standard Diet (SD). The WG diet was prepared by mixing autoclaved whole wheat berries with SD (50:50 w/w). The PG diet was prepared by mixing crushed matzo with SD (50:50 w/w). Mechanical testing confirmed significantly greater hardness for WG compared to PG

(two-tailed *t*-test; $p$=0.011). Fiber Bars (FB) and Placebo Bars (PB) were custom manufactured and mixed with SD (20:80 w/w).

***Inoculation.***

The polymicrobial inoculum consisted of *P. gingivalis* ($2.4\times10^8$), *T. denticola* ($2.4\times10^8$), *F. nucleatum* ($1\times10^8$), and *T. forsythia* ($1\times10^8$) cells/mL in 2% CMC in PBS.[23] Inoculum was prepared fresh daily and applied topically to molar quadrants using sterile micro-cotton swabs.

***Immunologic analysis via cytokine assay.***

Left hemi-mandibles were collected and gingival tissue was excised under 5× magnification. Tissue was homogenized in lysis buffer with protease inhibitor cocktail. IL-6, IL-23, IL-17A, IL-1β, IL-10, and TNF-α were quantified using the MILLIPLEX® Multiplex Assay using Luminex® technology. A table of six cytokines was imported into the phyloseq R package. Canberra distance matrices were constructed and PERMANOVA tests (adonis2 from the vegan package v2.6-2) were performed. Individual cytokine abundances were compared using Wilcoxon rank-sum tests.[36,37]

***Micro-computed tomography.***

Right hemimaxilla were fixed in 10% neutral buffered formalin and scanned using a Scanco Medical μCT50 at 10.0 μm resolution (55 kVP, 109 μA, 6W, 0.5 mm Al filter). Alveolar bone loss (ABC-CEJ distance) was measured at four sites (M1 crest, M1 furcation, M1/M2 interproximal, and M2 furcation) across three technical replicates per sample. Total bone loss was compared between diet and inoculation groups using a Wilcoxon rank-sum test.

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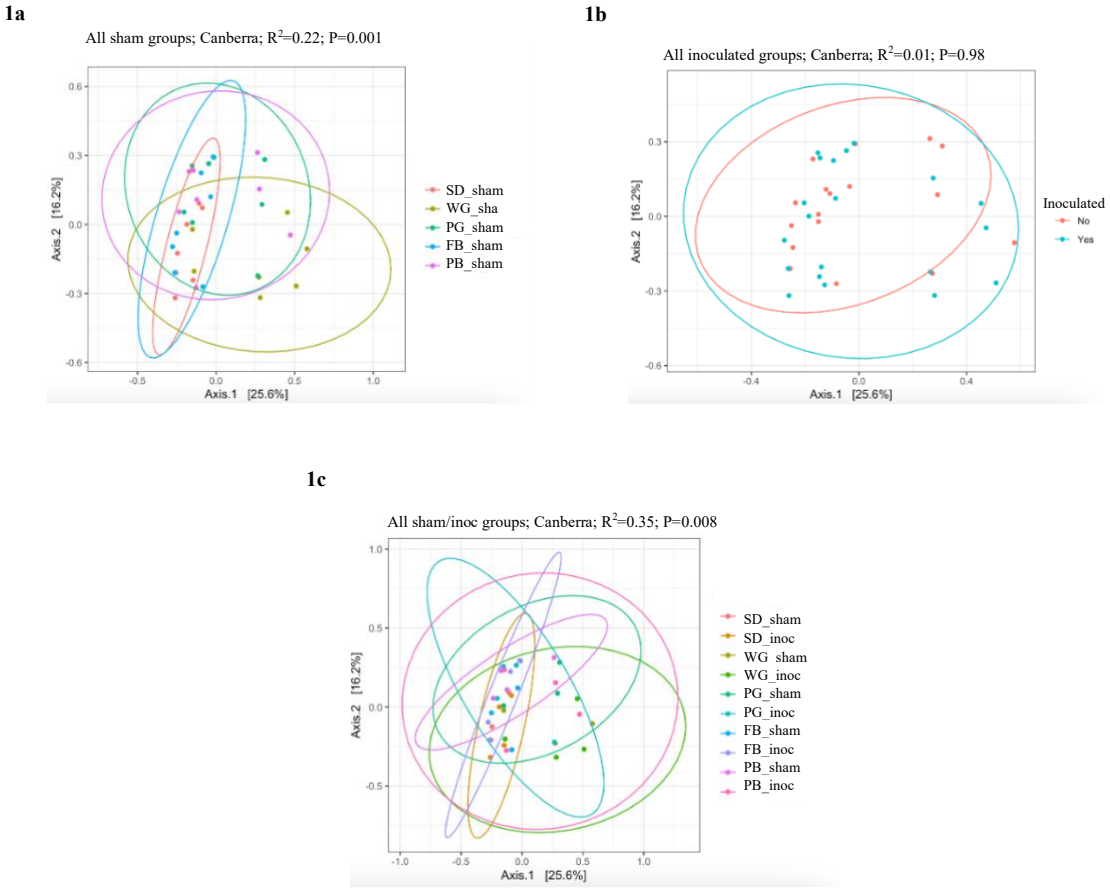
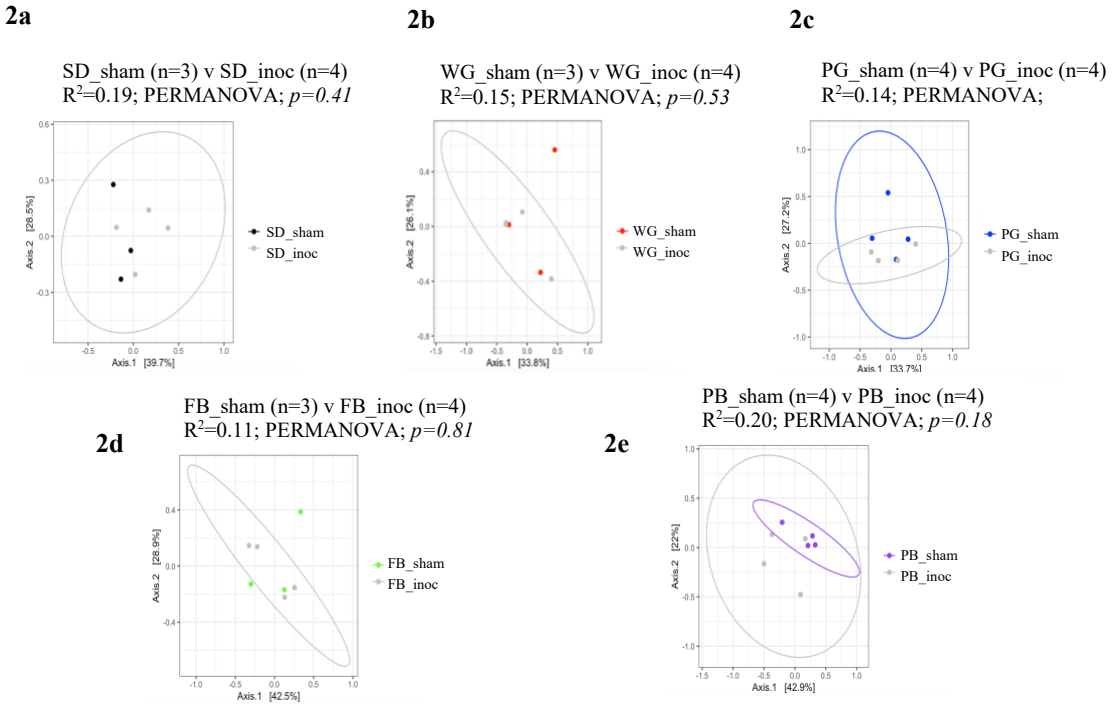
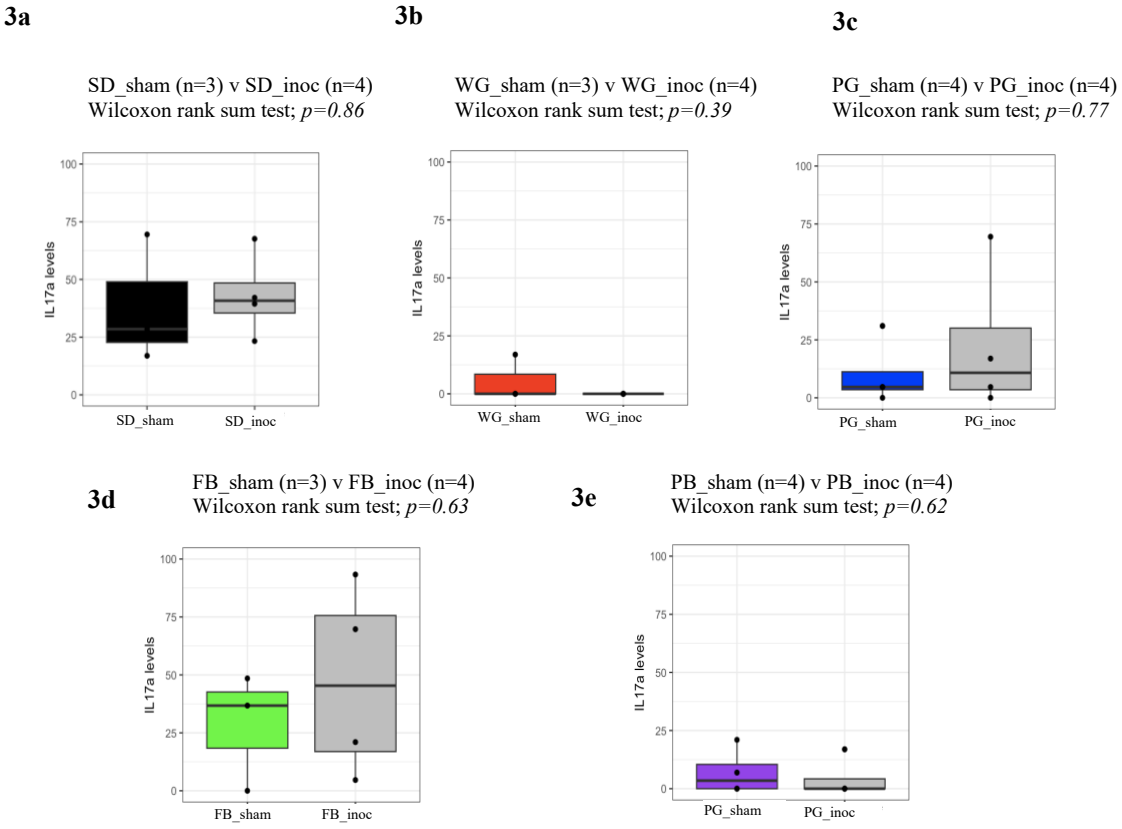
## Supplemental Figures



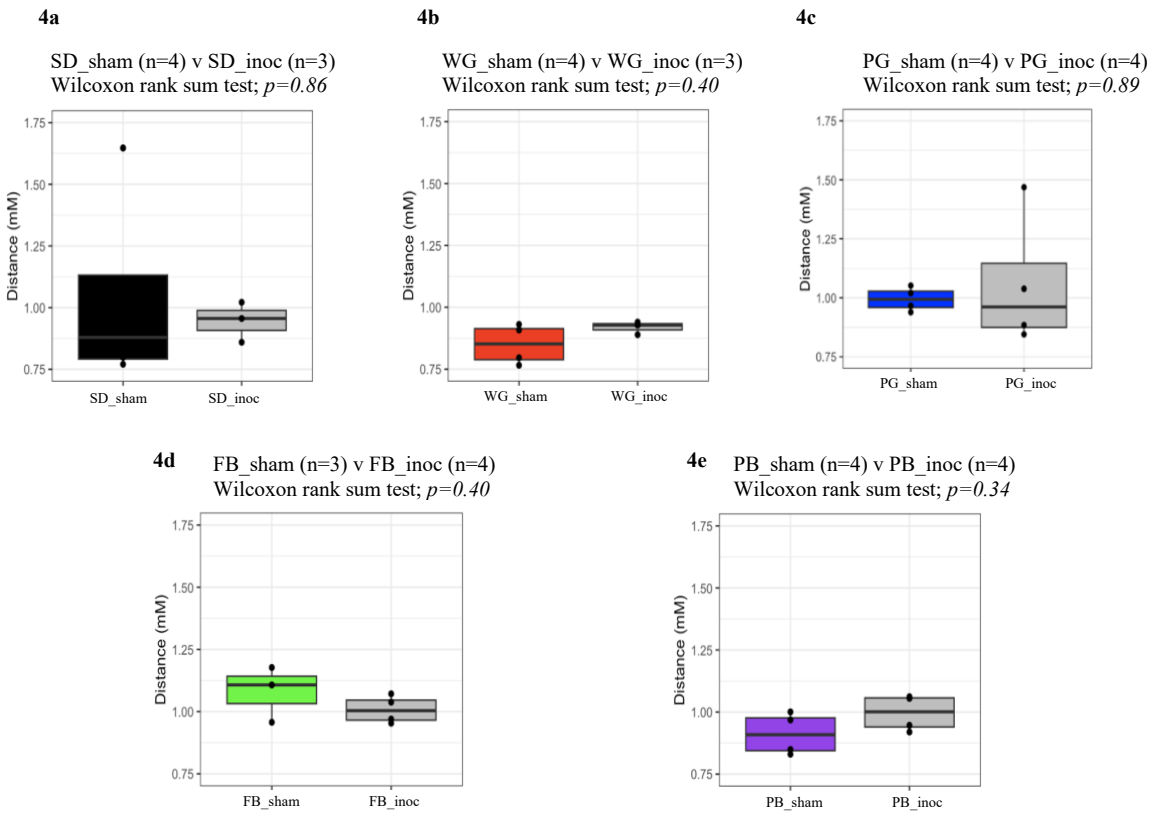

**Supplementary Fig. 3.1. Principal coordinates analysis of inflammatory cytokine profiles.** **1a.** Principal coordinates analysis (PCoA) using Canberra distance with samples grouped by diet (standard, whole grain, processed grain, fiber bar, placebo bar) (PERMANOVA, $R^2 = 0.22$, $P = 0.001$). **1b.** PCoA of the same samples grouped by inoculation status (PERMANOVA, $R^2 = 0.01$, $P = 0.98$). **1c.** PCoA with samples grouped by combined diet and inoculation status (PERMANOVA, $R^2 = 0.35$, $P = 0.008$). Ellipses indicate 95% confidence intervals.



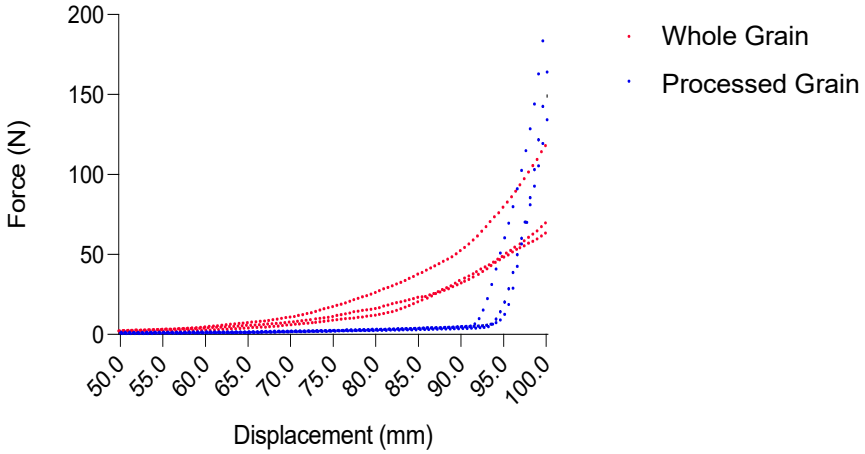

**Supplemental Fig. 3.2. Cytokine profile differences between sham and inoculated mice within each diet group.** Principal component analysis (PCoA) plots illustrate separation in overall cytokine expression profiles between sham and inoculated mice within the same dietary regimen: **2a.** standard diet (SD, black), **2b.** whole grain (WG, red), **2c.** processed grain (PG, blue), **2d.** fiber bar (FB, green), and **2e.** placebo bar (PB, purple). Each point represents an individual mouse, and ellipses indicate 95% confidence intervals for group clustering. Distinct clustering patterns between sham and inoculated groups suggest inoculation-induced shifts in cytokine expression, with the degree of separation varying across dietary conditions.




**Supplemental Fig. 3.3. IL-17A cytokine profiling between sham and inoculated mice within each diet group.** IL-17A concentrations were compared using the Wilcoxon rank sum test for each diet group, with colored boxplots representing sham and gray boxplots representing inoculated mice. Diet groups include **3a.** standard diet (SD, gray), **3b.** whole grain (WG, red), **3c.** processed grain (PG, cyan), **3d.** fiber bar (FB, green), and **3e.** placebo bar (PB, purple). No significant differences in IL-17A levels were observed between sham and inoculated groups for any of the diets: SD_sham vs SD_inoc (p = 0.86; 3a), WG_sham s WG_inoc (p = 0.39; 3b), PG_sham vs PG_inoc (p = 0.77; 3c), FB_sham vs FB_inoc (p = 0.63; 3d), and PB _sham vs PB_inoc (p = 0.62; 3e).

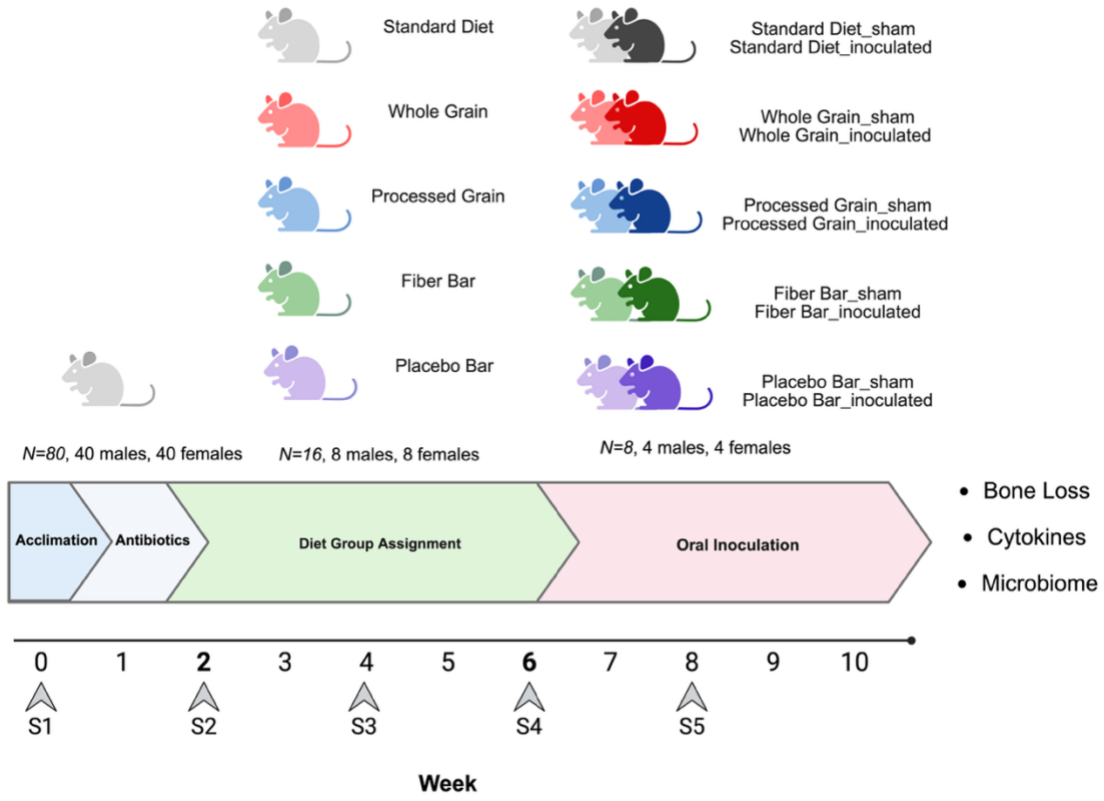



**Supplemental Fig. 3.4. Alveolar bone loss in sham vs. inoculated mice across dietary regimens.** Box plots illustrate alveolar bone loss, measured as the distance from the cemento-enamel junction to the alveolar bone crest (mm), in sham (colored) and inoculated (gray bars) mice across five dietary groups: standard diet (SD; black), whole grain (WG; red), processed grain (PG; cyan), fiber bar (FB; green), and placebo bar (PB; purple). Panels compare sham vs. inoculated mice within each diet group: **4a.** SD_sham vs. SD_inoc, **4b.** WG_sham vs. WG_inoc, **4c.** PG_sham vs. PG_inoc, **4d.** FB_sham vs. FB_inoc, **4e.** PB vs. PB_inoc. No significant increases in alveolar bone loss were observed in inoculated mice compared to their respective shams across all diet groups.




| | |
|---|---|
| Unpaired t test | |
| P value | 0.0114 |
| P value summary | * |
| Significantly different (P < 0.05)? | Yes |
| One- or two-tailed P value? | Two-tailed |
| t, df | t=2.555, df=200 |

**Supplemental Fig. 3.5. Food hardness of Whole Grain vs Processed Grain diets.** Load-displacement curves were generated to assess food hardness of custom diets composed of Whole Grain (red) or Processed Grain (blue) mixtures. Whole Grain diets exhibited significantly greater resistance to compression, indicating increased hardness compared to Processed Grain diets. Measurements were performed on n=6 samples per group.




**Supplemental Figure 3.6. Experimental design and timeline.** A schematic overview of the murine dietary and inoculated protocol is shown. **Week 0–1:** Mice underwent acclimation followed by oral antibiotic treatment and chlorhexidine rinse. **Week 2:** Mice were randomly assigned to one of five diet groups (n=16 per group; 8 males, 8 females): standard diet (gray), whole grain (red), processed grain (blue), fiber bar (green), or placebo bar (purple). **Week 6:** Mice were subdivided into inoculated (n=8; 4 males, 4 females) and sham groups (n=8; 4 males, 4 females). Inoculated groups were treated using a polymicrobial oral inoculation with *P. gingivalis*, *T. forsythia*, *T. denticola*, and *F. nucleatum*, while shams received a sham inoculation. **Week 10:** Final weights were collected, and hemi-maxillae and hemi-mandibles were harvested for analysis of alveolar bone loss, cytokines, and microbial composition. Oral swabs and stool were collected at five timepoints (S1–S5): S1 = pre-antibiotic, S2 = post-antibiotic, S3 = mid-diet, S4 = pre-inoculation, S5 = mid-inoculation.

## Chapter 4: Diet texture and fiber content regulate microbial community composition.

### Introduction

The oral cavity harbors one of the most diverse and dynamic microbial ecosystems in the human body, in which interactions among resident microorganisms and their host collectively determine states of oral health and disease.[1] As established in prior comprehensive reviews, the composition and functional capacity of these microbial communities are shaped by a constellation of genetic, environmental, and behavioral factors throughout life.[1,2] Among these determinants, diet occupies a particularly prominent position, serving simultaneously as a nutritional substrate for oral microorganisms and as a selective ecological pressure that enriches taxa best adapted to utilize available dietary resources. Landmark shifts in human dietary patterns, from pre-agricultural fibrous diets to the carbohydrate-dense, processed foods characteristic of modern Western diets, have been paralleled by profound restructuring of the oral microbiota, including the outgrowth of organisms implicated in dental caries and periodontal disease.[1,3]

While the influence of dietary carbohydrates on the oral microbiome has been well characterized,[4,5] the specific contributions of dietary fiber as a physical, mechanical force on dental surfaces, has received comparatively limited attention. Foundational work by Sedghi and colleagues using an *in vivo* murine model demonstrated that dietary fiber significantly restructures the dental microbiome, affecting both beta diversity at the phylum and genus levels and the relative abundance of key taxa.[6] That study further proposed that insoluble fiber may act primarily as an abrasive mechanical force on dental biofilm rather than as a direct metabolic contributor, given that the lignin used did not support microbial metabolism in the oral cavity. Critically, fiber supplementation reduced the relative abundance of genera such as *Streptococcus*, *Staphylococcus*,

and *Lactobacillus*, even in the concurrent presence of dietary sugar, suggesting a protective microbial effect attributable to the physical properties of fiber.[6] These findings were subsequently formalized into a reproducible methodological framework for characterizing the murine oral microbiome.[7]

The human oral microbiome is among the most extensively characterized microbial communities in the body, catalogued by the Human Oral Microbiome Database (HOMD), which currently encompasses more than 700 species-level phylotypes across 13 phyla.[8] The dominant phyla in the healthy human oral cavity, Firmicutes, Proteobacteria, Bacteroidetes, Actinobacteria, and Fusobacteria, are distributed in a site-specific manner across the diverse microenvironments of the oral cavity, including the supragingival plaque, subgingival sulcus, dorsal tongue, buccal mucosa, and saliva.[8,9] Each niche supports a compositionally distinct community whose membership reflects local differences in oxygen tension, pH, nutrient availability, host epithelial adhesion ligands, and immune surveillance. The subgingival microbiome is of relevance to periodontal disease, as it is enriched in Gram-negative anaerobic taxa, including members of the Bacteroidetes, Spirochaetes, and Fusobacteria phyla, whose virulence factors actively subvert host innate immunity and drive inflammatory alveolar bone destruction.[10,11]

A critical consideration in the interpretation of murine oral microbiome studies is the substantial compositional divergence between the mouse and human oral microbiota. The laboratory mouse oral microbiome is markedly less diverse than its human counterpart, comprising approximately 103 mouse oral taxa (MOT) spanning only four phyla, Firmicutes, Proteobacteria, Actinobacteria, and Bacteroidetes, compared to the more than 700 phylotypes across 13 phyla catalogued in humans.[12] The murine oral cavity is dominated by a relatively small number of taxa, with laboratory mice exhibiting particularly low species-level richness. Streptococcal species,

most prominently *Streptococcus danieliae* (mouse oral taxon MOT10), can account for the majority of oral bacterial reads in some murine colonies, a dominance pattern with no direct parallel in the more compositionally even human oral microbiome.[12,13] Critically, the classical periodontal pathogens that define the dysbiotic human subgingival microbiome (*Porphyromonas gingivalis*, *Treponema denticola*, and *Tannerella forsythia*) are not indigenous members of the murine oral microbiota and are believed to colonize the mouse oral cavity only transiently following experimental inoculation.[13,14] This absence of endogenous periodontal pathogens means that spontaneous periodontal dysbiosis in laboratory mice occurs rarely and inconsistently without experimental manipulation, which both limits and defines the scope of what murine models can reveal about the diet-microbiome-periodontitis axis.

Mouse strain background profoundly influences susceptibility to experimental periodontitis and the nature of the associated oral microbiome response, a variable that carries direct implications for the interpretation of results from this study. Inbred mouse strains differ substantially in their immunological architecture, particularly in the Th1/Th2/Th17 balance of their adaptive immune responses, and this genetic immunological variation translates into dramatically different periodontal phenotypes following microbial challenge.[15,16] Seminal strain-susceptibility studies identified BALB/cByJ and BALB/cJ mice as among the most susceptible inbred strains to *P. gingivalis*-induced alveolar bone loss, whereas C57BL/6J, A/J, SJL/J, and 129/J mice are substantially resistant.[15,17] This susceptibility difference is attributed in large part to the Th2-skewed cytokine profile of BALB/c mice, which produces elevated IL-4, IL-10, and IL-13 in response to periodontal challenge. In contrast, C57BL/6 mice mount a predominantly Th1 response characterized by interferon-γ and IL-2 production that is relatively protective against osteoclastic bone loss.[15,16] The choice of BALB/cByJ mice in the present study was justified in

that this strain provides a permissive host context for diet-driven differences in periodontal outcomes to manifest, without requiring the ligature-induced mechanical trauma that is often necessary to induce periodontitis in more resistant backgrounds. Beyond strain-level immune differences, several anatomical and behavioral features of the laboratory mouse further distinguish the murine oral ecosystem from the human oral environment. For example, mice are coprophagic, which introduces a persistent gut microbial input to the oral cavity that has no equivalent in human physiology and contributes to the representation of intestinal-associated taxa, including *Lactobacillus*, *Faecalibacterium*, and *Bifidobacterium*, in murine oral swab and gingival samples. The presence of these taxa in murine oral microbiome datasets therefore reflects both oral colonization and transient fecal-oral transmission, complicating direct comparison with human oral community profiles.[12,18]

Despite these important compositional and ecological differences, the murine model retains compelling advantages for controlled dietary intervention studies of the kind described herein. The ability to precisely define and maintain dietary regimens under controlled housing conditions, standardizing macronutrient intake, food texture, fiber delivery form, and co-housing microenvironment, is not achievable in human dietary studies, where confounding by variable oral hygiene, salivary flow, genetic diversity, and background dietary complexity is substantial. The murine oral microbiome, though compositionally simpler than its human counterpart, remains responsive to dietary perturbations through the same ecological mechanisms such as differential substrate availability, mechanical biofilm disruption, and altered salivary compositions that govern human oral microbial community dynamics.[6,7,12] The conservation of these mechanistic pathways across host species means that findings regarding the directional influence of dietary texture and fiber content on microbial community structure in mice generate mechanistic hypotheses that may

be translated to human clinical populations, even where the specific taxa responding to dietary perturbation differ.

Building on this prior work, the present chapter investigates how diet texture and fiber content regulate oral microbial community composition in the context of a polymicrobial periodontal inoculation model. Specifically, we compare four dietary regimens, whole grain (WG), processed grain (PG), fiber bar (FB), and placebo bar (PB), across both sham and inoculated animal groups to disentangle the contributions of dietary formulation from those of polymicrobial challenge. We hypothesize that diet texture and fiber content, rather than inoculation status per se, are the primary drivers of oral microbial community structure. Principal coordinate analysis (PCoA) based on Canberra distance matrices, differential abundance analysis, and genus-level relative abundance profiling are employed to characterize diet-associated microbial shifts and to identify taxa whose abundance is selectively modulated by dietary composition. Collectively, this chapter extends prior foundational investigations into the diet–oral microbiome axis by providing a controlled, multi-diet comparison within the context of periodontal disease pathogenesis.

## Results

### *Oral microbial community composition is shaped by diet rather than inoculation status.*

Oral microbial community composition was assessed using principal coordinate analysis (PCoA) based on Canberra distance matrices to determine the effect of inoculated status on microbial community structure. Ordinations including all diet × inoculated groups revealed significant differences in overall microbial composition ($R^2 = 0.20$, $P = 0.001$; **Supplemental Fig.**). However, inoculated groups did not differ significantly from sham groups ($R^2 = 0.20$, $P > 0.05$; **Supplemental Fig. 4.1b**), and diet explained more of the variance than inoculation status

($R^2$=0.11; P=0.001; **Supplemental Fig. 4.1c**). Moreover, no significant differences in microbial community composition were observed between non-inoculated and inoculated groups within the same diet regimen (**Supplemental Fig. 4.2**): WG_sham vs WG_inoc (PERMANOVA; $p$>0.05, $R^2$=0.14; **Supplemental Fig. 4.2a**), PG_sham vs PG_inoc (PERMANOVA; $p$>0.05, $R^2$=0.14; **Supplemental Fig. 4.2b**), FB_sham vs FB_inoc (PERMANOVA; $p$>0.05, $R^2$=0.082; **Supplemental Fig. 4.2c**), and PB_sham vs PB_inoc (PERMANOVA; $p$>0.05, $R^2$=0.64; **Supplemental Fig. 4.2d**), consistent with assessment of alveolar bone loss and cytokine profiling observed in the previous chapter. Pairwise ordinations between diet groups within inoculated strata were therefore performed to investigate the role of diet in shaping microbial communities.

***Dietary fiber and texture are relevant to microbial community composition.***

Oral microbial community composition was next assessed to determine the effect of diet regimen on community structure (**Fig. 4.1**). Among sham animals, no significant differences in microbial community composition were observed between WG_sham and PG_sham groups (PERMANOVA, $p > 0.05$, $R^2 = 0.08$; **Fig. 4.1a**), nor between FB_sham and PG_sham groups (PERMANOVA, $p > 0.05$, $R^2 = 0.107$; **Fig. 4.1a**). Similarly, comparison of sham FB and PB groups did not reveal significant differences in microbial community composition (PERMANOVA, $p > 0.05$, $R^2 = 0.19$; **Fig. 4.1b**), nor among WG_sham and FB_sham groups (PERMANOVA, $p > 0.05$, $R^2 = 0.11$; **Fig. 4.1c**). Among inoculated animals, no significant differences were detected between WG_inoc and PG_inoc groups (PERMANOVA, $p > 0.05$, $R^2 = 0.09$; **Fig. 4.1d**) or between FB_inoc and PB_inoc groups (PERMANOVA, $p > 0.05$, $R^2 = 0.14$; **Fig. 4.1e**). In contrast, a significant difference in microbial community composition was observed when comparing WG_inoc and FB_inoc groups (PERMANOVA, $p = 0.015$, $R^2 = 0.15$; **Fig. 4.1f**),

indicating that whole grain and fiber bar diets were associated with distinct oral microbial communities under polymicrobial inoculation conditions.

To examine diet-associated effects independent of inoculated status, sham and inoculated animals were pooled. No significant differences in microbial community composition were observed between WG_All and PG_All groups (PERMANOVA, $p > 0.05$, $R^2 = 0.12$; **Fig. 4.1g**). In contrast, FB_All and PB_All groups demonstrated significantly different microbial community compositions (PERMANOVA, $p = 0.048$, $R^2 = 0.22$; **Fig. 4.1h**), suggesting that the presence or absence of dietary fiber influenced oral microbial community structure. Additionally, WG_All and FB_All groups exhibited a significant difference in microbial community composition (PERMANOVA, $p = 0.001$, $R^2 = 0.26$; **Fig. 4.1i**), suggesting that the form in which dietary fiber was administered (whole grain versus fiber blend) may also associate with distinct microbial community structures. Collectively, while dietary regimen alone did not consistently alter oral microbial community composition, the most pronounced differences were observed between whole grain and fiber bar diets under polymicrobial-inoculum treated (**Fig. 4.1f**) or pooled conditions (**Fig. 4.1i**).

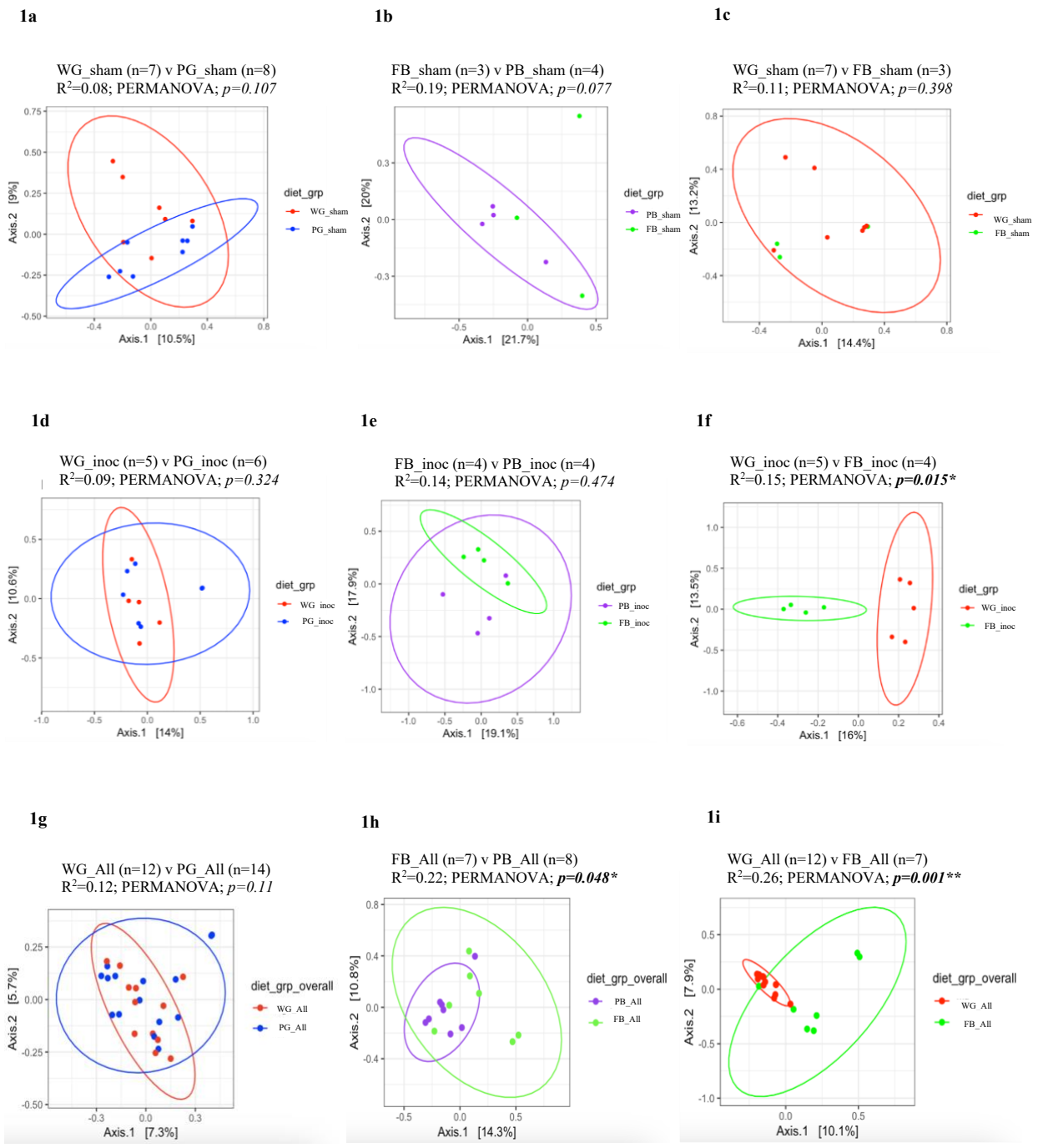



**Figure 4.1. Microbial community composition.** Principal coordinate analysis (PCoA) plots based on Canberra distance matrices visualize microbial community composition across dietary groups, in both sham and inoculated mice. Each panel shows the first two axes of variation, with ellipses representing 95% confidence intervals. Statistical differences between groups were assessed using PERMANOVA. **4.1a.** WG_sham (n=7) vs PG_sham (n=8): No significant difference observed ($R^2$=0.08, p=0.107). **Figure legend continued next page.**

**Figure legend continued from previous page. 4.1b.** FB_sham (n=3) vs PB_sham (n=4): Communities showed a trend toward separation ($R^2$=0.19, p=0.077). **4.1c.** WG_sham (n=7) vs FB_sham (n=3): No significant differences ($R^2$=0.11, p=0.398). **4.1d.** WG_inoc (n=5) vs PG_inoc (n=6): No significant separation ($R^2$=0.09, p=0.324). **4.1e.** FB_inoc (n=4) vs PB_inoc (n=4): $R^2$=0.14, p=0.474 (not significant). **4.1f.** WG_inoc (n=5) vs FB_inoc (n=4): Communities were significantly different ($R^2$=0.15, p=0.015*). **4.1g.** WG_All (n=12) vs PG_All (n=14): Overall differences not statistically significant ($R^2$=0.12, p=0.11). **4.1h.** FB_All (n=7) vs PB_All (n=8): Communities differed significantly ($R^2$=0.22, p=0.048*). **4.1i.** WG_All (n=12) vs FB_All (n=7): Significant separation observed ($R^2$=0.26, p=0.001**). *Asterisks indicate statistical significance at $p < 0.05$; double asterisks indicate $p < 0.01$.*

***Relative abundance is designated by diet rather than inoculation status.***

To assess the impact of inoculated status on genus-level microbial composition within each dietary regimen, relative abundance profiles of inoculated and sham animals were compared (**Supplemental Fig. 4.3**). Within the whole grain (WG) diet, WG_sham communities were characterized by a higher relative contribution of *Lactobacillus*, whereas WG_inoc communities showed greater relative contributions of *Faecalibacterium* and *Enterococcus* (**Supplemental Fig. 4.3a**). However, differential abundance analysis of the top ten genera did not identify any statistically significant differences between inoculated states (Wilcoxon rank-sum test, $p > 0.05$). Similarly, within the processed grain (PG) diet, PG_sham samples were characterized by *Enterococcus*, while PG_inoc samples exhibited a greater relative contribution of *Staphylococcus* (**Supplemental Fig. 4.3b**). No statistically significant differences in the relative abundance of the top ten genera were observed between inoculated states (Wilcoxon rank-sum test, $p > 0.05$). Comparison of inoculated and sham groups within the fiber bar (FB) and placebo bar (PB) diets revealed broadly similar community compositions dominated by *Enterococcus* and *Staphylococcus* (**Supplemental Fig. 4.3 c and d**), with no significant differences between inoculated states (Wilcoxon rank-sum test, $p > 0.05$). Collectively, these findings indicate that

inoculation status did not significantly alter the relative abundance of dominant genera within any dietary group.

***Diet texture and fiber content elicit significant differences in relative abundance of specific genera.***

Genus-level community composition differed across dietary conditions in sham animals (**Fig. 4.2**). WG_sham samples were primarily characterized by a high relative abundance of *Lactobacillus*, whereas PG_sham samples were characterized by a greater relative contribution of *Enterococcus* (**Fig. 4.2a**). *Enterococcus* (zero-inflated negative binomial modeling, $P_{adj}$ = 5.76 E -5), *Bifidobacterium* (zero-inflated negative binomial modeling, $P_{adj}$ = 4.48 E -4), and *Delftia* (zero-inflated negative binomial modeling; $P_{adj}$ = 7.73 E -5) differed significantly between WG_sham and PG_sham groups. Comparison of FB_sham and PB_sham groups revealed broadly similar overall community structures; both groups were dominated by *Enterococcus* (**Fig. 4.2b**), with significant enrichment of *Staphylococcus* identified among PB_sham groups (zero-inflated negative binomial modeling, $P_{adj}$ = 0.029). Comparison of WG_sham and FB_sham groups further highlighted diet-associated differences: WG_sham communities were characterized by *Lactobacillus*, whereas FB_sham samples exhibited increased relative contributions of *Enterococcus* and *Staphylococcus* (**Fig. 4.2c**). Significant enrichment of *Bifidobacterium* was observed among FB_sham groups compared to WG_sham (zero-inflated negative binomial modeling, $P_{adj}$ = 1.96 E -103).

Among inoculated animals, WG_inoc samples were characterized by prominent contributions of *Enterococcus* and *Faecalibacterium*, whereas PG_inoc samples showed greater relative representation of *Pseudomonas* and *Delftia* (**Fig. 4.3a**) with significant enrichment of *Agathobacter* identified among PB_inoc groups (zero-inflated negative binomial modeling, $P_{adj}$ =

3.45 E -18). Both FB_inoc and PB_inoc groups were characterized by substantial relative abundance of *Enterococcus* and *Staphylococcus* observed (**Fig. 4.3b**), with significant enrichment of *Staphylococcus* observed among PB_inoc (zero-inflated negative binomial modeling, $P_{adj}$ = 0.048). Comparison of WG_inoc and FB_inoc groups identified *Staphylococcus* as significantly enriched among FB_inoc groups (zero-inflated negative binomial modeling, $P_{adj}$ = 0.012) (**Fig. 4.3c**).

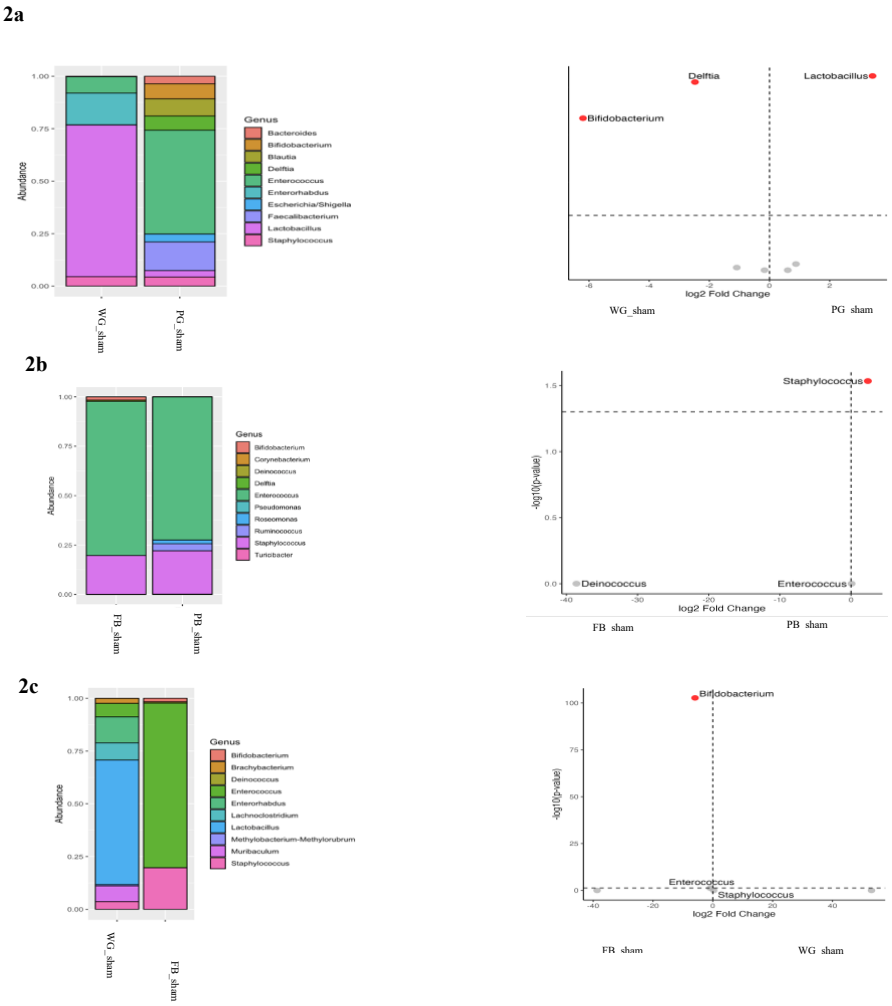



**Figure 4.2. Relative abundance among sham groups.** Stacked bar plots depict mean genus-level relative abundance across dietary conditions in sham mice. Corresponding volcano plots (right panels) display differential abundance results for the same pairwise comparison, with $\log_2$ fold change plotted against statistical significance. **4.2a.** WG_sham communities were primarily characterized by *Lactobacillus*, whereas PG_sham communities were characterized by *Enterococcus*. Significant enrichment of Lactobacillus ($p = 0.0000576$) was observed among WG_sham and significant enrichment of Delftia ($p = 0.0000773$) and Bifidobacterium ($p = 0.000448$) was observed among PG_sham. **4.2b.** FB_sham and PB_sham communities were both dominated by *Enterococcus and Staphylococcus. Significant enrichment of Staphylococcus (p adj = 0.029) was observed among PB_sham groups* **4.2c.** WG_sham was characterized by *Lactobacillus*, while FB_sham showed greater relative contributions of *Enterococcus* and *Staphylococcus*. Significant enrichment of *Bifidobacterium* was observed among FB_sham groups compared to WG sham (p adj = 1.96 E -103).

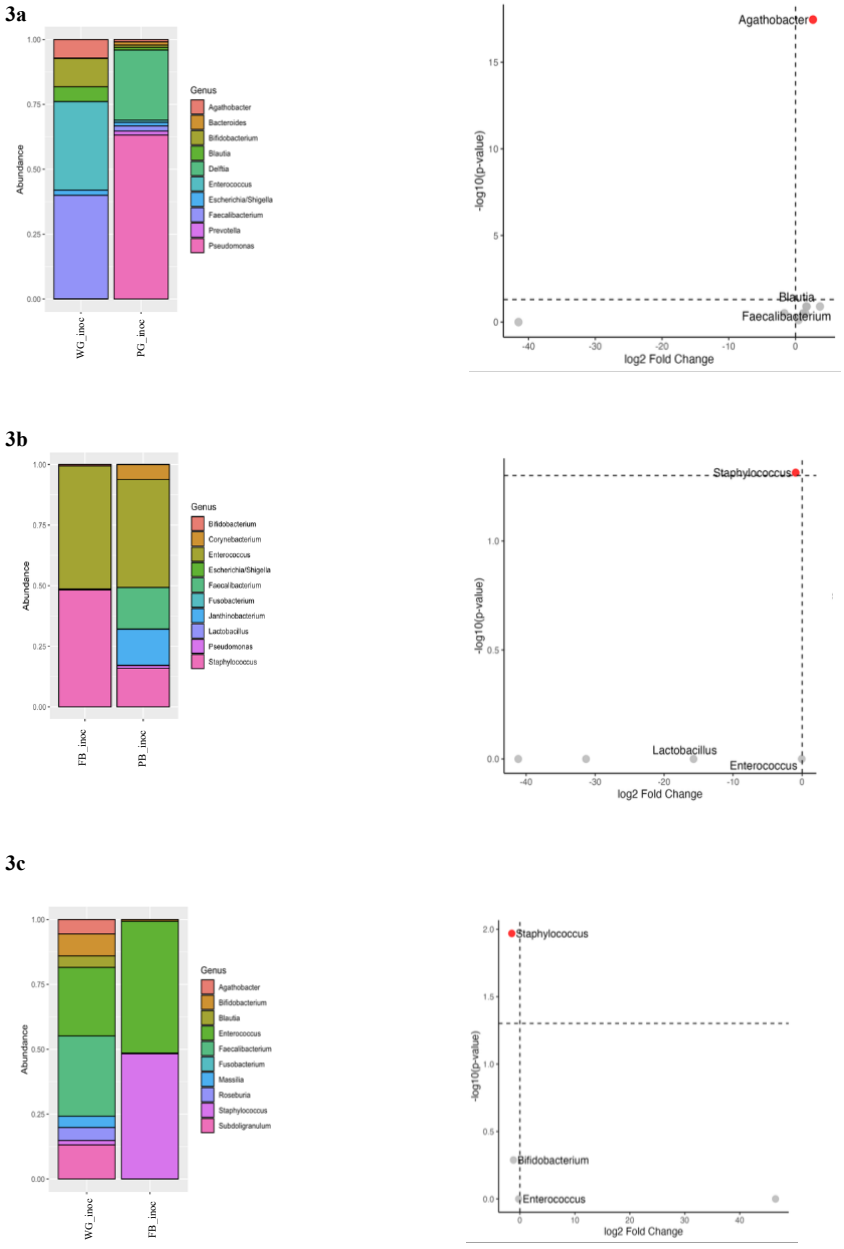


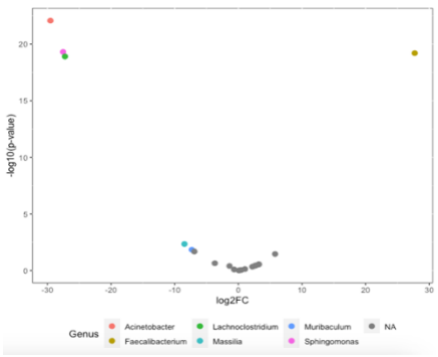

**Figure 4.3. Relative abundance among inoculated groups.** Stacked bar plots depict genus-level relative abundance across dietary conditions in inoculated mice and corresponding volcano plots depict significant genera identified using zero-inflated negative binomial testing. **4.3a.** WG_inoc and PG_inoc groups exhibited distinct compositional profiles, with WG_inoc characterized by *Enterococcus* and *Faecalibacterium* and PG_inoc characterized by *Pseudomonas* and *Delftia*. *Agathobacter* was significantly enriched among PG_inoc groups (p adj = 3.45 E -18) **4.3b.** FB_inoc and PB_inoc communities were both dominated by *Enterococcus* and *Staphylococcus*. Staphylococcus was significantly enriched among PB_inoc groups (p adj = 0.048) **4.3c.** WG_inoc was characterized by *Enterococcus* and *Faecalibacterium*, whereas FB_inoc showed increased relative contribution of *Staphylococcus*.

***Differential abundance of genera is shaped by diet texture as well as fiber content.***

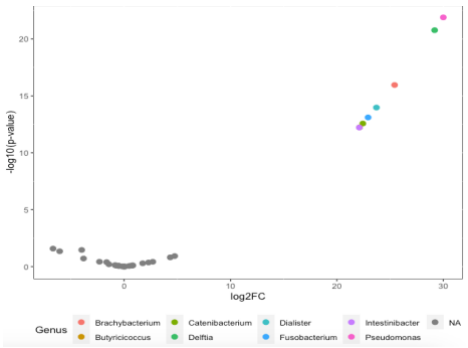
Differential abundance analysis using DESeq2 evaluated inoculation-associated microbial shifts within each dietary regimen (**Supplemental Fig. 4.4**). Within the WG diet, *Lachnoclostridium* was significantly enriched among WG_sham animals whereas *Subdoligranulum* was significantly enriched in WG_inoc animals (adjusted $p < 0.05$; **Supplemental Fig. 4.4a**). Within the PG diet, widespread inoculation-associated shifts were observed, including significant enrichment of *Prevotella*, *Brachybacterium*, *Moraxella*, *Acinetobacter*, *Dialister*, *Fusobacterium*, *Dorea*, *Catenibacterium*, *Intestinibacter*, and *Butyricicoccus* in PG_inoc animals (adjusted $p < 0.05$; **Supplemental Fig. 4.4b**). Within the FB diet, *Deinococcus* was the only genus with statistically significant differential abundance (enriched in FB_sham; adjusted $p < 0.05$; **Supplemental Fig. 4.4c**). No genera with statistically significant differences were identified in the PB diet following correction.

Diet-associated differential abundance analysis among sham animals (**Fig. 4.4a-c**) identified multiple genera distinguishing WG from PG conditions, including *Acinetobacter* (enriched in WG_sham) and *Faecalibacterium*, *Sphingomonas*, *Lachnoclostridium*, *Massilia*, and *Muribaculum* (enriched in PG_sham). In FB vs PB sham comparisons, *Deinococcus* was enriched in FB_sham and *Staphylococcus* in PB_sham. Among inoculated animals (**Fig. 4.4d-f**), *Pseudomonas*, *Delftia*, *Brachybacterium*, *Dialister*, *Fusobacterium*, *Catenibacterium*, *Butyricicoccus*, and *Intestinibacter* were significantly enriched in PG_inoc relative to WG_inoc. *Subdoligranulum*, *Blautia*, *Acinetobacter*, *Methylobacterium–Methylorubrum*, and *Dorea* were significantly enriched in WG_inoc relative to FB_inoc, collectively demonstrating that dietary composition strongly modulated microbial community structure among inoculated animals.

**4a** DESeq2 WG_sham (n=6) v PG_sham (n=8)

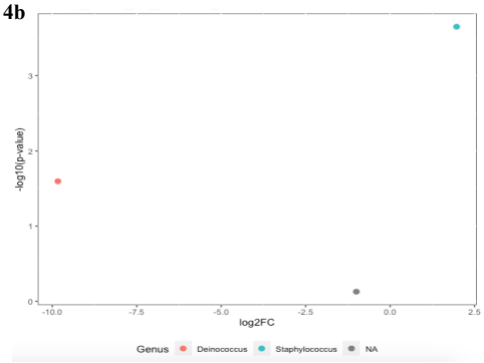



**4d** DESeq2 WG_inoc (n=5) v PG_inoc (n=7)



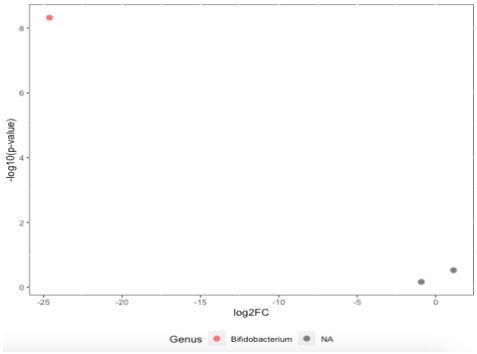

DESeq FB_sham (n=3) v PB_sham (n=2)

**4b**



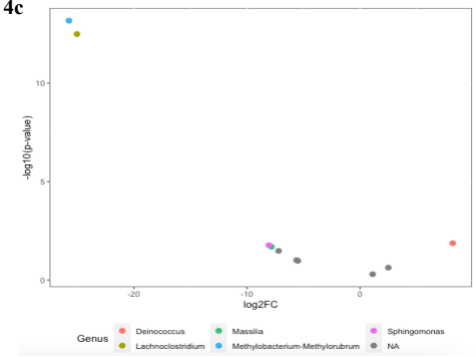

DESeq2 FB_inoc (n=4) v PB_inoc (n=2)

**4e**




DESeq WG_sham (n=6) v FB_sham (n=3)

**4c**




DESeq2 WG_inoc (n=5) v FB_inoc (n=4)

**4f**

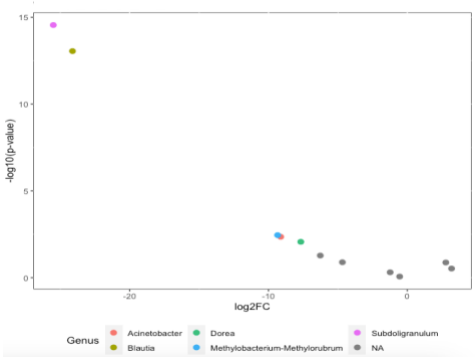



**Figure 4.4. Differential abundance analysis among dietary groups.** Volcano plots depict genus-level differences in microbial abundance across dietary groups in sham mice. **4.4a.** WG_sham (n = 6) vs PG_sham (n = 8) **4.4b.** FB_sham (n = 3) vs PB_sham (n = 2) **4.4c.** WG_sham (n = 6) vs FB_sham (n = 3) **4.4d.** WG_inoc (n = 5) vs PG_inoc (n = 7) **4.4e.** FB_inoc (n = 4) vs PB_inoc (n = 2) **4.4f.** WG_inoc (n = 5) vs FB_inoc (n = 4). Dashed lines on volcano plots indicate the adjusted significance threshold (padj = 0.05).

## Discussion

The principal finding of this chapter is that oral microbial community composition in BALB/cByJ mice is shaped primarily by dietary regimen rather than by polymicrobial periodontal inoculation status. Across multiple analytical frameworks, including beta diversity ordination, genus-level relative abundance comparisons, and DESeq2 differential abundance analysis, diet consistently accounted for more variance in community structure than microbial challenge, and pairwise comparisons within each dietary group revealed no significant differences between sham and inoculated animals. This pattern, which parallels the findings from cytokine profiling and alveolar bone loss analyses reported in Chapter 3, argues for the primacy of diet as an ecological determinant of the oral microbiome in this experimental context.[6,7,19]

Within the dietary comparisons, diet texture emerged as the more consistent and potent driver of microbial community differentiation relative to fiber content. The most statistically robust and ecologically informative differences in community composition were observed between the WG and FB groups, diets that were generally comparable in total dietary fiber content but differed fundamentally in the structural form of fiber (WG provided intact grain texture through the preservation of the fibrous bran-encapsulated grain architecture, whereas FB delivered fiber in a homogeneous, soft supplemental form). The significant differences in beta diversity observed between WG and FB under pooled conditions and between WG_inoc and FB_inoc, contrasted with the absence of significant community-level differences between FB and PB groups, suggest that the fiber-associated community differences observed in this study were driven by the textural properties of intact grain rather than by the presence or absence of fiber supplementation.[6,19,20]

The mechanistic basis for texture-mediated microbial community regulation operates through at least two complementary pathways. First, the masticatory mechanical forces generated

by intact grain texture impose shear and compressive forces on dental biofilm, disrupting its maturation and limiting the sequential colonization that drives community succession toward dysbiotic states.[20,21] Hard dietary substrates are known to reduce biofilm abundance on dental surfaces, and this biofilm-limiting effect selectively disadvantages taxa that depend on a mature biofilm architecture for colonization, including many of the Gram-negative anaerobes enriched in the processed grain diet groups. Second, intact grain texture stimulates salivary flow and composition, increasing the delivery of antimicrobial peptides, secretory IgA, bicarbonate, and salivary amylase to the oral cavity.[22] Salivary amylase also preferentially hydrolyzes exposed starch in processed grain compared to the fiber-encapsulated starch of intact whole grain, generating differential saccharide substrate availability that shapes the metabolic ecology of the supragingival biofilm.[4,5]

At the genus level, the dietary differences in community composition observed in this study reveal ecologically meaningful patterns relevant to periodontal disease pathogenesis. The enrichment of *Lactobacillus* in WG groups, relative to the higher relative representation of *Enterococcus* and *Staphylococcus* in PG and FB groups, is consistent with prior murine dietary fiber studies.[6] *Lactobacillus*, while often characterized as saccharolytic, includes species with demonstrated inhibitory activity against periodontal pathogens via bacteriocin and organic acid production.[23] In contrast, *Enterococcus* and *Staphylococcus* represent taxa associated with dysbiotic oral states and opportunistic infection, and their enrichment in texturally soft diet groups may reflect reduced biofilm disruption and increased fermentable substrate availability under processed grain conditions.[24] The pronounced enrichment of *Fusobacterium* and *Prevotella*, in PG_inoc relative to WG_inoc animals is noteworthy in that *Fusobacterium nucleatum* serves as a bridging species in the succession from early to late periodontal pathogens,[10] and *Prevotella*

species are recognized as accessory pathogens in the dysbiotic subgingival microbiota of human periodontitis.[11] The selective enrichment of these genera under processed grain plus inoculation conditions, but not under whole grain plus inoculation, suggests that diet texture may determine the ecological permissiveness of the oral environment for the establishment of inoculated periodontal pathogens.

The compositional divergence between the murine and human oral microbiomes discussed in the Introduction requires careful consideration when interpreting the translational relevance of these findings. The taxa observed in this study, including *Lactobacillus*, *Enterococcus*, *Staphylococcus*, *Faecalibacterium*, and *Deinococcus*, are not the primary constituents of the human periodontal microbiome, which is dominated by Streptococcaceae, Veillonellaceae, Prevotellaceae, Fusobacteriaceae, and Pasteurellaceae.[8,9] The dominance of *Enterococcus* and *Staphylococcus* in the processed grain and fiber bar groups may in part reflect the murine oral ecological context, including the contribution of coprophagy to oral microbial composition, rather than a community shift that would have direct parallels in the human subgingival microbiome. Nevertheless, the directional finding that dietary texture selectively enriches commensals and suppresses opportunistic taxa is mechanistically interpretable in terms of the ecological plaque hypothesis and has functional parallels in human dietary microbiome studies showing that whole grain and high-fiber diets shift the oral microbiome toward compositional profiles associated with better periodontal health.[3,25]

A further notable finding is that the processed grain diet seemingly amplified the microbiome response to polymicrobial inoculation. Among all dietary groups, PG was the only condition in which the periodontal inoculum produced statistically significant DESeq2-detected genus-level shifts, including the enrichment of *Fusobacterium* in PG_inoc animals. This finding

may suggest that the processed grain diet creates a microbiome that is ecologically primed for exogenous pathogen colonization, a "dysbiosis-permissive" state, whereas the textured whole grain diet confers a degree of ecological resilience that limits the compositional impact of periodic pathogen challenge.[10,19]

Several important limitations of this study must be acknowledged. First and most fundamentally, the use of a murine model introduces a systematic compositional gap between the oral microbiome characterized here and the human oral microbiome of clinical relevance. The murine oral microbiota is substantially less diverse than the human oral microbiota, is dominated by murine-specific taxa absent from the Human Oral Microbiome Database (HOMD), and lacks the indigenous periodontal pathogen community that drives dysbiosis in human periodontitis.[8,12] The classical periodontal pathogens introduced via oral lavage in this study, including *P. gingivalis*, *T. denticola*, *T. forsythia*, and *F. nucleatum*, are not resident members of the murine oral microbiota[13,14] which likely explains why inoculation status accounted for relatively little variance in community composition compared to diet in that the duration of inoculation was insufficient. The extent to which the diet-driven microbial community differences observed in mice correspond to specific compositional shifts in the human oral microbiome, particularly in the subgingival compartment relevant to periodontitis, remains to be empirically established.

Second, the 16S rRNA V4 amplicon sequencing approach employed here provides taxonomic resolution to the genus level in most cases but cannot resolve species-level diversity or capture functional capacity. The ecological significance of genus-level community differences; for example, whether an increase in *Enterococcus* relative abundance reflects a shift toward virulent vs. commensal species, cannot be assessed without species-level or metagenomic resolution.

Future studies incorporating shotgun metagenomic sequencing would provide both higher taxonomic resolution and the functional gene content data needed to determine whether diet-associated community shifts are accompanied by changes in the metabolic potential of the oral microbiome.[26]

Third, sample sizes per group for microbiome analysis (n=4 per condition for deep analysis) are modest, limiting statistical power for the detection of subtle community-level differences. The stringency of the DESeq2 multiple testing correction (Benjamini-Hochberg FDR) applied in the differential abundance analyses may have resulted in conservative estimates of the number of differentially abundant genera, particularly in comparisons where trends were observed without reaching adjusted significance thresholds.[27]

## Methods

Methods for study design, diet preparation, antibiotic treatment phase, inoculation, and oral swab and stool collection are detailed in Chapter 3.

### *16S rRNA Sequencing and Analysis.*

#### *DNA Extraction.*

Left hemimaxillae were collected and stored in 1.5 mL cryovials, flash-frozen in liquid nitrogen, and stored at −80°C. Gingival tissue was carefully excised from hemi-maxillae on sterile, chilled petri dishes using a sterile microsurgical blade. DNA was extracted from gingival tissues using the DNEasy® Powersoil® Pro Kit (QIAGEN®) per the manufacturer's instructions, with slight modifications: frozen gingival tissue was added directly to the PowerBead Pro® tube, 800 µL of CD1 solution was added, and samples were incubated at 65°C for 10 minutes. Samples were secured horizontally on a vortex adaptor and vortexed at maximum speed (3200 RPM) for 30 minutes. Samples were centrifuged and supernatant was transferred to a clean 2 mL microcentrifuge tube. Extracted DNA was stored in C6 elution buffer at −20°C until further processing.

#### *Amplicon library preparation, sequencing, and data processing.*

DNA concentrations were quantified using a Qubit dsDNA Broad Range kit and normalized to 5 ng/µL. The V4 region of the 16S rRNA gene was amplified using barcoded 515F and 818R primers. PCR reactions (25 µL) were performed under the following thermal cycling conditions: 98°C denaturation (2 min), followed by 35 cycles of 98°C (20 s), 50°C (30 s), 72°C (45 s), and final extension at 72°C (10 min). Amplicons were normalized, pooled in equimolar concentrations, purified, and sequenced on an Illumina MiSeq platform (2×300 bp paired end

reads). Reads were demultiplexed by barcode using QIIME.[28] Pre-processing and taxonomic assignment against the Silva v138 database were performed using the DADA2 package.[29] ASVs with <0.0001% total read count across all samples were removed. Negative control filtering was performed by removing ASVs present in >15% of negative controls and <15% of samples. Amplicon library preparation and sequencing were conducted twice, resulting in two technical replicates; only overlapping ASVs were retained and mean read counts were calculated. Data underwent variance-stabilizing transformation using DESeq2.[27]

***Statistical analysis.***

To examine differences in microbial composition between diet and inoculation groups, a Canberra distance matrix was constructed using the distance function from phyloseq (v1.38.0).[30] PERMANOVA tests (adonis2 from the vegan package v2.6-2) were performed.[31] Relative abundance plots were generated to identify and visualize the top ten genera. Differential abundance analysis was performed using DESeq2 to detect taxa with significant log2 fold changes between groups, with p-values adjusted for multiple comparisons using Benjamini-Hochberg FDR correction.[27]

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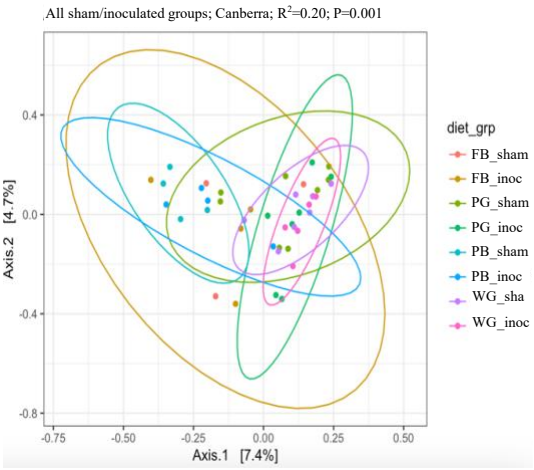
**1a**

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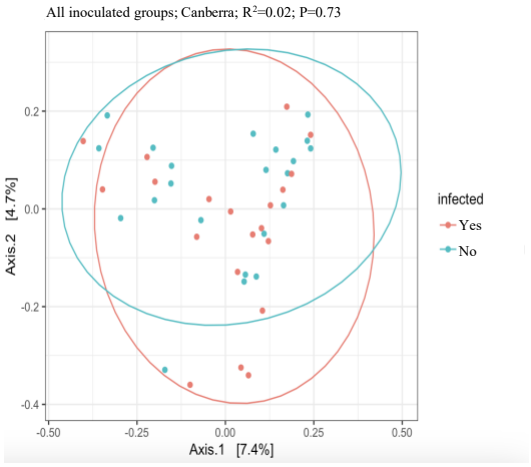


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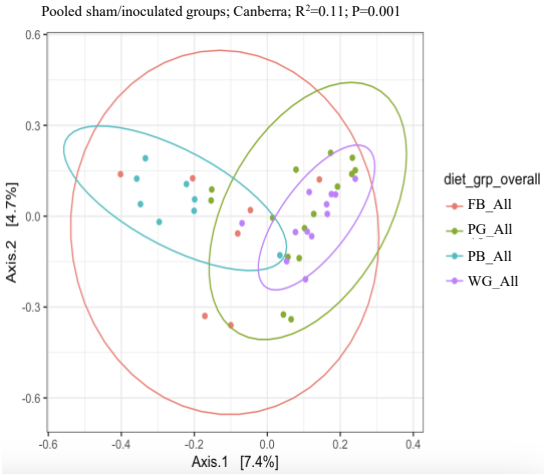
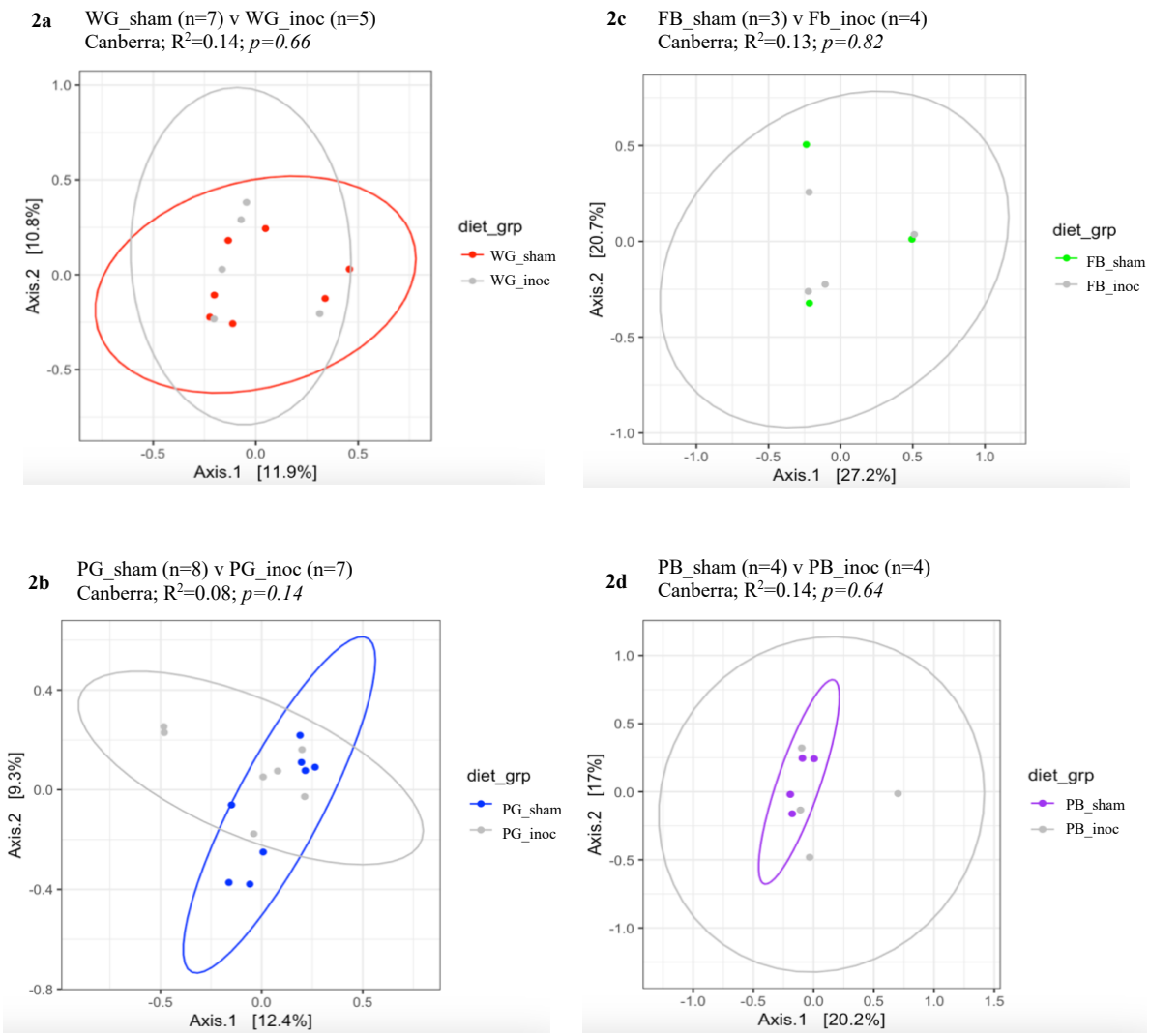
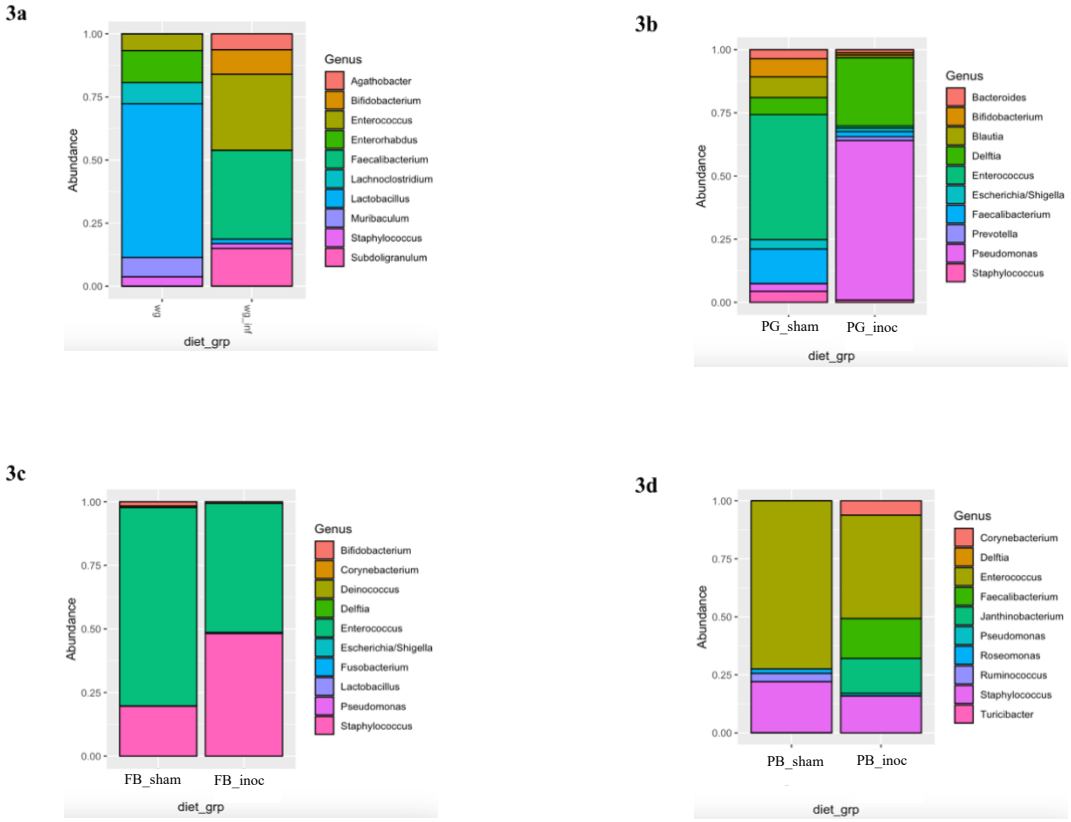



**Supplemental Fig. 4.1. Principal coordinates analysis of global microbial community composition**. **4.1a.** Principal coordinates analysis (PCoA) using Canberra distance with samples grouped by individual diet groups (PERMANOVA, $R^2 = 0.20$, $P = 0.001$). **4.1b.** PCoA of the same samples grouped by inoculation status (PERMANOVA, $R^2 = 0.02$, $P = 0.73$). **4.1c.** PCoA with samples grouped by overall diet category (WG, PG, FB, PB) (PERMANOVA, $R^2 = 0.11$, $P = 0.001$). Ellipses indicate 95% confidence intervals.

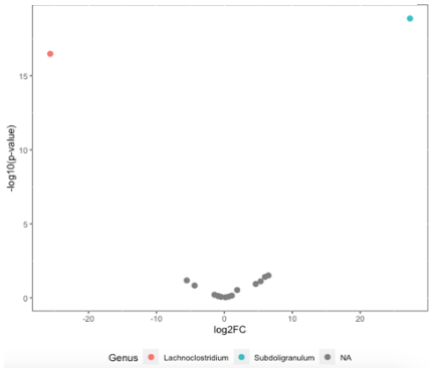



**Supplemental Fig. 4.2. Microbial community composition within diet groups.** Principal coordinate analysis (PCoA) plots depict two-dimensional ordinations based on Canberra distance matrices to assess microbial community structure between sham and inoculated mice within each dietary group. Each point represents an individual mouse, and ellipses indicate 95% confidence intervals for group clustering. Comparisons are shown for: **4.2a.** Whole grain (WG_sham, red) vs. WG_inoc (gray), **4.2b**. Fiber bar (FB_sham, green) vs. FB_inoc (gray), **4.2c**. Processed grain (PG_sham, blue) vs. PG_inoc (gray), **4.2d**. Placebo bar (PB_sham, purple) vs. PB_inoc (gray). Canberra R and p-values reflect the degree of microbial community separation between sham and inoculated groups for each diet. No comparisons reached statistical significance.

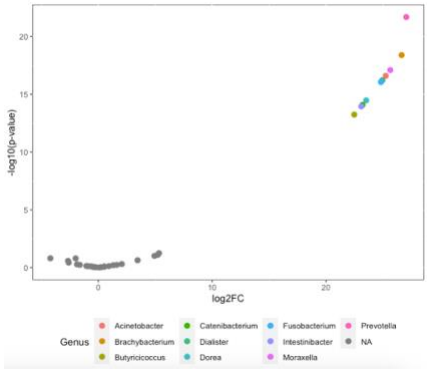



**Supplemental Fig. 4.3. Relative abundance of top bacterial genera in inoculated vs. sham mice across dietary regimens.** Stacked bar plots display the relative abundance of the top ten bacterial genera in sham (left bars) and inoculated (right bars) mice for each diet. whole grain (**4.3a**), processed grain (**4.3b**), fiber bar (**4.3c**), and placebo bar (**4.3d**). Each genus is represented by a distinct color. Wilcoxon rank sum tests revealed no statistically significant differences in the relative abundance of the top ten genera between inoculated states across any diet ($p > 0.05$).

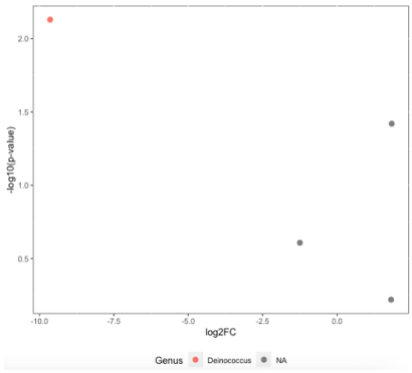
**4a** DESeq2 WG_sham (n=6) v WG_inoc




**4b** DESeq2 PG sham (n=8) v PG inoc




**4c** DESeq2 FB sham (n=3) v FB inoc




**Supplemental Fig. 4.4. Differential abundance analysis for sham vs inoculated groups.** Volcano plots) depict microbial shifts between sham (noninf) and inoculated (inf) animals within each dietary group. **4.4a.** WG_sham (n=6) vs WG_inoc (n=5). **4.4b.** PG_sham (n=8) vs PG_inoc (n=7): **4.4c.** FB_sham (n=3) vs FB_inoc (n=4).

# Chapter 5: Conclusion

## Summary of Findings

The present thesis investigated the mechanistic pathways by which dietary texture and fiber content regulate periodontal host-microbe homeostasis using a controlled murine model of experimental periodontitis. Across three experimental chapters, the collective data converge on a coherent and internally consistent conclusion: dietary texture, the physical-mechanical property conferred by intact grain architecture and fibrous food structure, emerged as the dominant dietary modifier of periodontal outcomes, and its effects are not recapitulated by fiber supplementation in the absence of food texture. This conclusion is supported by convergent evidence across three independent outcome measures: oral biofilm abundance and architecture, gingival inflammatory cytokine profiles and alveolar bone morphology, and oral microbial community composition.

In Chapter 2, the foundational mechanistic premise of this thesis through complementary in vivo and in vitro experiments were established, demonstrating that food particle size, as a proxy for grain processing, directly regulates oral biofilm abundance and growth kinetics. Animals fed a hard pellet diet accumulated substantially less biofilm on dental surfaces than those consuming a soft powdered equivalent, despite identical nutritional composition.[1,2] In vitro, fine-particle grain digests, reflective of increased amylolytic accessibility of processed grain starch following removal of the fibrous bran encapsulation,[3,4] supported significantly greater oral bacterial growth kinetics across all digestion time intervals tested, and produced biofilms with nearly double the surface area of those grown in coarse-particle digests. Taken together, these findings identified two synergistic pathways through which grain processing may disrupt periodontal homeostasis: removal of the masticatory mechanical challenge that limits biofilm maturation, and augmentation of fermentable saccharide substrate availability to pioneer oral colonizers.[5,6]

These mechanistic observations were then extended to in vivo host immune and bone phenotypes in Chapter 3, in which mice were subjected to five dietary regimens in the presence and absence of polymicrobial periodontal inoculation. Diet, rather than inoculation status, emerged as the dominant explanatory variable for both gingival cytokine profiles and alveolar bone loss across all analytical comparisons.[7,8] Whole grain (WG)-fed animals exhibited significantly lower alveolar bone loss and reduced gingival IL-17A levels compared to animals consuming either processed grain (PG) or fiber bar (FB) diets, with no significant differences observed between FB and placebo bar (PB) groups which differed only in the presence or absence of fiber, respectively. The positive correlation between gingival IL-17A and total alveolar bone loss across all dietary groups provided mechanistic support for IL-17A as a plausible intermediate through which dietary texture exerts its protective effects on periodontal bone integrity.[9,10]

Chapter 4 characterized the oral microbial community composition across dietary groups using 16S rRNA V4 amplicon sequencing and multivariate community analyses. Consistent with the immune and bone outcomes of Chapter 3, diet accounted for more variance in microbial community structure than inoculation status, and pairwise comparisons within each dietary group revealed no significant compositional differences between sham and inoculated animals.[11,12] The most ecologically and statistically robust community-level differences were observed between WG and FB groups, in which diets differed fundamentally in the structural form of fiber delivery. The WG diet selectively enriched *Lactobacillus*, while PG and FB diets were characterized by greater relative representation of *Enterococcus* and *Staphylococcus*. Among inoculated animals, processed grain diets uniquely supported enrichment of *Fusobacterium* and *Prevotella*—bridging and accessory periodontal pathogens, respectively, suggesting that dietary texture may determine

the ecological permissiveness of the oral environment for the establishment of periodontitis-associated taxa.[13,14]

**Dietary Texture as the Dominant Dietary Modifier of Periodontal Homeostasis**

The central hypothesis of this thesis, that being dietary texture is the dominant dietary modifier of periodontal host-microbe homeostasis, and that this textural effect is not fully recapitulated by fiber supplementation in the absence of food texture, is supported by the pattern of results across all experiments. The WG versus PG comparison, which simultaneously varies grain texture and intrinsic fiber content through grain refinement, consistently produced significant differences in biofilm biology, inflammatory cytokine profiles, alveolar bone loss, and microbial community composition. In contrast, the FB versus PB comparison, which isolates the contribution of fiber supplementation in the absence of textural distinction, failed to produce significant differences across nearly all outcome measures. The WG versus FB comparison, which compares the presence of fiber varying its structural form, consistently produced the most pronounced and statistically robust differences observed in this thesis, reinforcing the interpretation that the protective effects of whole grain consumption are attributable principally to the masticatory mechanical properties of intact grain texture rather than to its fiber content per se.[1,7,11]

This conclusion is biologically grounded in at least two complementary mechanistic pathways. First, fibrous grain texture imposes mechanical shear and compressive forces on dental surfaces during mastication that directly disrupt biofilm maturation and limit the sequential ecological succession that drives communities toward dysbiotic states.[2,15] Dental hard tissues are non-shedding surfaces that lack the epithelial renewal mechanisms available to mucosal surfaces, rendering them constitutively dependent on externally applied mechanical forces for biofilm limitation.[5,6] The present in vivo data demonstrating reduced biofilm burden on the dental surfaces

of hard-diet animals are consistent with epidemiological observations that populations consuming predominantly abrasive, fibrous diets exhibit lower rates of periodontal disease, even in the absence of contemporary oral hygiene practices.[16,17]

Second, intact grain texture stimulates masticatory mechanosensory input to gingival epithelial cells, which in turn drives a homeostatic IL-17 signaling axis mediated by IL-6-dependent activation of resident Th17 cells and γδ-T cells at the gingival barrier.[18] This physiological IL-17 response, elicited independently of microbial dysbiosis and IL-23 signaling, reinforces gingival barrier competency through upregulation of β-defensins, S100 alarmin proteins, and CXCL chemokines, priming an immunological readiness state without initiating destructive inflammation.[18,19] The loss of dietary texture with grain refinement interrupts this mechanosensory circuit, potentially depriving gingival tissues of the homeostatic inflammatory tone required to maintain barrier integrity and resist dysbiosis-driven pathological Th17 expansion. The present data, demonstrating lower steady-state gingival IL-17A and reduced alveolar bone loss in WG animals, are consistent with a model in which whole grain texture sustains this homeostatic axis, while processed grain and soft fiber supplementation fail to do so.[9,10,18]

**Distinguishing the Contributions of Fiber Content and Food Texture**

A critical methodological contribution of this thesis is its experimental design, which varied dietary texture and fiber content as distinct parameters within a single controlled framework. Prior epidemiological and interventional studies linking dietary fiber intake to periodontal health have generally been unable to disentangle the contributions of fiber content from the structural and textural properties of fiber-rich foods, since high-fiber diets are typically also texturally harder and lower in refined carbohydrates.[20,21,22] The present multi-diet design addresses this gap by comparing WG and PG diets, which simultaneously vary texture and fiber

through grain refinement, with FB and PB diets, which vary fiber content independently of food texture. This design enables a controlled dissociation of mechanistic pathways that observational studies cannot achieve.

The finding that fiber supplementation in the absence of grain texture (FB diet) did not significantly protect against alveolar bone loss, gingival IL-17A elevation, or dysbiotic microbial community shifts relative to the placebo bar control argues against a purely metabolic model of the protective effect of dietary fiber on periodontal health. Under such a model, fiber supplementation would be expected to reduce fermentable carbohydrate availability to the oral microbiota and shift the biofilm ecology toward commensal-dominated states.[23,24] While such effects may operate over longer dietary intervention periods or at higher fiber doses than those tested here, the present data suggest they are insufficient, in isolation, to replicate the magnitude of protection conferred by intact grain texture. Dietary fiber's oral health benefits appear to require its physical embodiment in an intact, fibrous food matrix that generates masticatory mechanical challenge, rather than its chemical identity as a non-digestible polysaccharide.[1,7]

This interpretation aligns with the foundational work of Sedghi and colleagues, which demonstrated that insoluble lignin-based fiber, a substrate with minimal oral fermentability, significantly restructured the murine dental microbiome, an effect attributed to physical-mechanical rather than metabolic mechanisms.[11,12] The present thesis extends this mechanistic framework to a broader set of dietary conditions and multiple outcome domains, providing convergent evidence that the structural properties of dietary fiber are central to its oral health effects. This has important implications for the interpretation of clinical fiber supplementation trials, which may underestimate the oral health potential of whole grain diets by supplying fiber in

homogeneous, soft supplemental forms that do not recapitulate the textural properties of intact grain.[20,21]

**Diet as an Ecological Determinant: Primacy Over Microbial Challenge**

A consistent finding across Chapters 3 and 4 is that dietary regimen, not polymicrobial inoculation status, explained the preponderance of variance in gingival cytokine profiles, alveolar bone outcomes, and oral microbial community composition. Within each dietary group, sham and inoculated animals were statistically indistinguishable across nearly all outcome measures, while cross-diet comparisons consistently produced significant differences. This pattern argues for the primacy of diet as an ecological determinant of the oral environment, one that operates upstream of periodic microbial challenge in shaping the baseline inflammatory set point of gingival tissues and the ecological structure of the oral microbiome.[7,11]

This finding is consistent with the ecological plaque hypothesis and its extensions, which conceptualize periodontal disease as a community-level ecological disturbance driven by environmental perturbations rather than by simple host-pathogen infection.[5,6] Under this framework, diet functions as a continuous ecological pressure that shapes biofilm composition through substrate availability and mechanical disruption, and shapes host immune tone through mechanosensory and metabolic signals. The inoculated periodontal pathogens—*Porphyromonas gingivalis*, *Treponema denticola*, *Tannerella forsythia*, and *Fusobacterium nucleatum*—may act as ecological amplifiers of an already diet-determined microbiome trajectory rather than as independent disease-initiating agents. Notably, PG was the only dietary condition in which inoculation produced statistically significant DESeq2-detected genus-level shifts, including enrichment of *Fusobacterium*, suggesting that processed grain diet creates a microbiome that is ecologically primed for exogenous pathogen colonization, a state permissive to dysbiosis, whereas

intact grain texture confers a degree of microbial resilience that limits the compositional impact of periodic pathogen challenge.[13,14]

**Translational Implications**

The findings of this thesis have several translational implications for the prevention and adjunctive management of periodontal disease. Current dietary guidance for periodontal health has focused primarily on the reduction of refined carbohydrates and fermentable sugars, and on the adoption of anti-inflammatory dietary patterns characterized by increased omega-3 fatty acids, dietary antioxidants, and fiber intake.[20,22,25] The present data support this guidance but add a mechanistically important qualification: the physical form of dietary fiber matters as much as, or more than, its chemical quantity. Clinical guidance that encourages increased dietary fiber intake without specifying the importance of intact, texturally challenging food structures, such as whole grains, raw vegetables, fibrous fruits, may fail to capitalize on the texture-mediated mechanical and immunological pathways identified here.

Grain refinement, which simultaneously removes dietary fiber and reduces food texture, may represent the key dietary insult linking Western dietary patterns to elevated periodontal disease prevalence.[3,4,26] The confluence of increased fermentable saccharide availability and reduced masticatory challenge creates dual conditions permissive for oral biofilm dysbiosis: a substrate-enriched environment that supports saccharolytic pioneer species and their dysbiotic successors, and the absence of the mechanical disruption and mechanosensory immune priming that limit biofilm maturation and maintain gingival barrier integrity. This dual insult model, supported by the convergent data of this thesis, provides a mechanistic rationale for the superior oral health profiles observed in populations consuming pre-agricultural fibrous diets[16,17] and in

contemporary human dietary intervention studies substituting whole grain for refined grain consumption.[21,22]

From a clinical standpoint, these findings suggest that dietary counseling for patients with periodontal disease or elevated periodontal risk should explicitly address food texture as a modifiable oral health determinant, distinct from and complementary to conventional oral hygiene instruction. The substitution of refined grain products with structurally intact whole grain alternatives, such as wheat berries, whole grain breads with intact bran, oats, and similar foods, may confer oral health benefits through both the reduction of fermentable carbohydrate bioavailability and the restoration of masticatory mechanical stimulation of gingival tissues. Such recommendations are consistent with established guidelines for the prevention of non-communicable diseases more broadly, and their adoption could contribute to periodontal health as a co-benefit of dietary improvements motivated by systemic health goals.[25,27]

**Limitations and Considerations**

Several important limitations of this thesis must be acknowledged when interpreting its findings and their translational scope. The use of a murine experimental model introduces a systematic compositional gap between the oral microbiome characterized here and the human oral microbiome of clinical relevance. The murine oral microbiota is substantially less diverse than its human counterpart, is dominated by murine-specific taxa absent from the Human Oral Microbiome Database, and lacks the indigenous periodontal pathogen community that drives dysbiosis in human periodontitis.[28,29] The inoculated periodontal pathogens are not resident members of the murine oral microbiota and colonize the mouse oral cavity only transiently, which likely accounts for the limited impact of inoculation status on community composition and immune phenotypes observed across experimental chapters. The extent to which the diet-driven microbial and immune

differences identified in mice correspond to specific compositional and immunological shifts in the human oral cavity—particularly in the subgingival compartment relevant to clinical periodontitis—remains to be empirically established.

Sample sizes per group were modest (n=4 per dietary-inoculation condition for deep immunological and microbiome analyses), limiting statistical power for the detection of subtle group differences and necessitating cautious interpretation of comparisons that achieved significance only after pooling of sham and inoculated animals. Replication in larger, appropriately powered cohorts will be required to confirm the directionality and magnitude of the effects reported here, particularly for the exploratory WG versus FB comparisons.

The cytokine multiplex panel, while targeted to key periodontitis-associated mediators, captured only six cytokines and did not include amphiregulin, β-defensins, RANKL, OPG, or the full complement of chemokines implicated in the mechanosensory-barrier immune circuit. The 16S rRNA V4 amplicon approach provides genus-level taxonomic resolution but cannot resolve species-level diversity or functional capacity, limiting interpretation of the ecological significance of observed genus-level shifts. Future studies incorporating broader transcriptomic or proteomic tissue profiling, shotgun metagenomic sequencing, and precisely defined synthetic diets would substantially advance the mechanistic resolution of the diet–periodontal homeostasis axis.[28,29]

**Future Directions**

The findings of this thesis generate several high-priority questions for future investigation. First, the mechanosensory-immune circuit identified in the gingival barrier, in which dietary texture drives IL-6-dependent homeostatic IL-17 production by Th17 cells and γδ-T cells to reinforce epithelial barrier function,[18,19] warrants direct characterization in the context of the dietary manipulations employed here. Future studies should quantify gingival γδ-T cell

populations and their Vγ subset composition across dietary groups using flow cytometry and spatial immunofluorescence and assess the expression of barrier-reinforcing mediators including amphiregulin, β-defensins, and S100 proteins. Establishing whether the WG diet expands gingival γδ-T cells and sustains their amphiregulin and IL-17 co-production, while PG and FB diets fail to do so, would provide direct cellular evidence for the mechanosensory pathway proposed here.

Second, translation of the murine dietary texture findings to human populations requires carefully designed clinical studies that can independently vary food texture while controlling for fiber content and macronutrient composition. A crossover dietary intervention comparing intact whole wheat berries with matched refined wheat flour products, both incorporated into standardized diets with equivalent total fiber, protein, and caloric content, would test whether the texture-mediated effects observed in mice are conserved in human periodontal tissues. Outcome measures would include gingival crevicular fluid cytokine profiling, supragingival and subgingival microbiome characterization, and non-invasive assessments of gingival barrier integrity alongside standard clinical periodontal indices.[20,21,22]

Third, the observation that the processed grain diet created a microbiome ecologically primed for exogenous pathogen colonization warrants further mechanistic dissection. Future experiments employing germ-free or gnotobiotic mouse models with defined microbial communities could directly test whether processed grain diets facilitate stable colonization of introduced periodontal pathogens in a manner that whole grain diet prevents and could identify the microbial and host immune mechanisms that underlie this ecological resistance.[13,14]

**The Irreversibility of Periodontitis and the Imperative for Prevention**

A critical but underappreciated feature of periodontitis is that the tissue destruction it produces is, for practical purposes, irreversible. Unlike gingivitis, a reversible inflammatory

response confined to the gingival soft tissues that resolves fully with effective plaque control,[7,8] periodontitis is defined by attachment loss and alveolar bone destruction, structural changes that cannot be recovered without surgical intervention and are never fully restored even with it.[7,32] Once the periodontal ligament has been disrupted and alveolar bone resorbed, the regenerative capacity of the periodontium is insufficient to rebuild the lost architecture spontaneously. Guided tissue regeneration, bone grafting, and other surgical modalities can slow further destruction and partially reconstitute lost attachment in select cases, but they do not restore patients to a pre-disease state, carry significant morbidity and cost, and are unavailable to large segments of the population due to access and financial barriers.[32,33] The endpoint of uncontrolled periodontitis, that being edentulism, is irreversible. Tooth loss eliminates the affected organ permanently, with cascading consequences for mastication, nutrition, speech, facial aesthetics, and psychosocial wellbeing that persist for the remainder of the patient's life.[34,35]

Given this irreversibility, prevention, not treatment, represents the only strategy capable of preserving the full periodontal apparatus across a lifetime. Yet the contemporary dental healthcare system in the United States and most high-income countries remains overwhelmingly oriented toward the treatment of established disease rather than the prevention of its onset. Periodontal therapy, including scaling and root planing, surgical pocket reduction, and implant rehabilitation of edentulous patients, accounts for a disproportionate share of dental expenditure relative to preventive services.[33,36] While professional cleanings and patient education in oral hygiene are standard components of routine dental care, dietary counseling specific to periodontal risk is rarely incorporated into dental practice guidelines or reimbursement structures. The result is a system that manages the consequences of periodontal disease with escalating interventional intensity

while failing to address the upstream dietary and environmental conditions that determine who develop the disease and at what age.[36,37]

This prevention gap is especially consequential given the epidemiological scale of periodontal disease. Severe periodontitis affects approximately 11% of the global population, making it the sixth most prevalent disease worldwide, and moderate-to-severe forms affect nearly half of American adults over age 30.[38,39] Prevalence increases steeply with age, but the biological processes that ultimately produce adult periodontitis, including dysbiotic biofilm maturation, gingival barrier compromise, early alveolar bone changes, begin accumulating well before clinical disease is detected and, in many patients, well before adulthood. The recognition that periodontitis is the downstream consequence of chronic ecological and immunological disruption that begins early in life reframes prevention not as a matter of adult dental hygiene habits alone, but as a lifecycle challenge demanding early and sustained dietary and behavioral intervention.[7,40]

The findings of this thesis contribute directly to this prevention argument. If dietary texture is a dominant determinant of the gingival immune set point and oral microbial ecology, operating continuously and cumulatively throughout the lifetime of the host, then the dietary environments to which individuals are exposed beginning in childhood represent a long-horizon determinant of their adult periodontal health trajectories. A child raised on a diet dominated by processed, texturally soft refined grains may accumulate years of mechanosensory deficit at the gingival barrier, progressive biofilm dysbiosis, and subthreshold inflammatory damage before any clinical sign of periodontal disease is detectable. By the time the disease is identified and referred for periodontal treatment, irreversible attachment loss may already have occurred. Prevention must therefore begin before disease is present—and ideally before the dietary patterns that promote it are firmly established.

**Implications for Pediatric Dentistry**

Pediatric dentistry has historically focused its preventive mandate on dental caries, a condition that shares etiological roots with periodontitis in its dependence on saccharolytic oral biofilm and fermentable carbohydrate substrate, but that manifests earlier in childhood and has therefore commanded greater preventive attention in the pediatric context.[41,42] Periodontal disease, by contrast, is typically conceptualized as an adult condition, and pediatric dental encounters rarely include substantive assessment of periodontal risk factors beyond basic gingival inflammation screening. This framing is increasingly difficult to sustain considering evidence that the oral microbiome, gingival immune architecture, and dietary patterns that determine long-term periodontal susceptibility are established during childhood and adolescence.[40,43]

The oral microbiome undergoes its most dynamic compositional transitions in early childhood, when the dentition erupts and the supragingival and subgingival niches are first colonized by the taxa that will constitute the resident microbiota for decades.[43] Dietary texture and composition during this critical window shape the ecological conditions under which pioneer communities establish, and may exert disproportionate influence on the long-term compositional trajectory of the oral microbiome relative to dietary exposures later in life. Children who consume predominantly soft, processed food during the period of primary and mixed dentition may establish dysbiosis-permissive biofilm communities that persist into adolescence and adulthood, setting an elevated baseline risk for periodontal disease later in life.[40,41]

Moreover, the mechanosensory-immune circuit identified in gingival tissues is responsive to masticatory mechanical stimulation from the onset of dental eruption. The gingival γδ-T cell network that mediates homeostatic IL-17 production and barrier reinforcement in response to dietary hardness[18,19] must be established and maintained through ongoing mechanical challenge if

it is to function as intended. Children raised on diets that fail to provide adequate masticatory challenge during the primary and mixed dentition periods may not develop the full complement of gingival γδ-T cells and Th17 cells required for mature barrier homeostasis, leaving their periodontia constitutively under-defended against dysbiotic challenge. Whether such developmental deficits in the mechanosensory-immune circuit are recoverable through later dietary change, or whether they produce lasting alterations in gingival immune architecture, is an important and uninvestigated question with direct relevance to pediatric dietary intervention timing.

These considerations argue for the expansion of periodontal risk assessment and dietary counseling into routine pediatric dental care in a manner that parallels existing caries prevention protocols. Just as pediatric dentists counsel caregivers on the reduction of dietary sugar exposure to limit cariogenic biofilm, they are well positioned to counsel families on the importance of dietary texture, the regular consumption of whole grains, raw fruits and vegetables, and other fibrous foods, as a complementary strategy for maintaining gingival health and limiting the early oral dysbiosis that precedes adult periodontitis. Such counseling need not require additional clinical time if integrated into the dietary history and anticipatory guidance frameworks already employed in pediatric dental practice.[41,42] The present mechanistic data provide an evidence base for such guidance that previously did not exist: dietary texture now has a defined biological pathway through which it protects the periodontium, making it a defensible target for clinical dietary recommendation.

Importantly, pediatric dental encounters occur with a frequency and during developmental windows that make them uniquely valuable contact points for preventive intervention. In the United States, children covered by Medicaid are entitled to dental preventive services beginning

at age one, and commercially insured children typically receive twice-yearly dental examinations through adolescence.[36] These visits represent structured, repeated opportunities to assess dietary patterns, counsel families, and reinforce behaviors that will determine oral health decades into the future. The current failure to leverage these encounters for periodontal prevention, beyond basic gingival health inspection, represents a missed opportunity of substantial magnitude.

**Dietary Policy and Government-Level Intervention for Pediatric Populations**

The evidence linking grain refinement and soft dietary texture to periodontal disease susceptibility has implications that extend beyond the clinical encounter to the policy environments that shape what children eat. Dietary patterns in pediatric populations are not solely the product of individual family choices; they are powerfully shaped by institutional food environments including school meal programs, childcare nutrition standards, food assistance programs, and agricultural policy that are subject to government regulation and are therefore potential levers for population-level dietary change.[44,45]

In the United States, the National School Lunch Program (NSLP) and School Breakfast Program (SBP) serve approximately 30 million children daily and represent the dominant institutional dietary exposure for a large fraction of the pediatric population, particularly among children from low-income households who are at disproportionate risk for both poor dietary quality and poor oral health outcomes.[44,46] Federal nutrition standards for these programs, administered by the United States Department of Agriculture (USDA), already mandate minimum whole grain requirements for grain-based items served in participating schools. However, compliance with these standards is uneven, enforcement mechanisms are limited, and the practical implementation of whole grain requirements frequently substitutes whole wheat flour for refined flour in otherwise texturally soft products such as breads that do not reconstitute the masticatory mechanical

challenge of intact grain.[44,45] The present mechanistic data suggest that this approach, while perhaps more nutritionally beneficial in terms of fiber content delivery, may not confer the texture-mediated periodontal protective effects of genuinely intact grain structures. Policy standards that specify not only the grain content of school foods but their textural properties, thus incentivizing the inclusion of intact grain products, minimally processed whole grain foods, and raw fruits and vegetables with meaningful masticatory demand, would more faithfully translate the oral health benefits of dietary texture into institutional food environments.

The Special Supplemental Nutrition Program for Women, Infants, and Children (WIC) presents an analogous opportunity for early intervention. WIC provides food package benefits to approximately 6.2 million low-income pregnant women, new mothers, infants, and children up to age five, precisely the population in which early dietary texture exposures may have the greatest influence on the developmental trajectory of the oral microbiome and gingival immune architecture.[46] WIC food packages have undergone meaningful nutritional revision in recent decades, with the 2009 and 2020 revisions substantially increasing fruit, vegetable, and whole grain components.[45,46] Building on this progress by further emphasizing intact, texturally challenging grain foods and minimizing highly processed grain-based items within WIC-eligible food categories would align program benefits with emerging evidence on the texture-mediated determinants of periodontal health.

**Economic Burden of Periodontitis and Edentulism: The Case for Prevention Investment**

The economic argument for population-level periodontal prevention is substantial and has been insufficiently incorporated into health policy deliberations about dietary standards, dental benefit design, and public health resource allocation. Periodontal disease and its sequela, tooth loss and edentulism, generate direct healthcare costs through the clinical management of disease, and

indirect costs through reduced workforce productivity, nutritional compromise, and the downstream treatment of associated systemic conditions.[33,36,48]

Direct dental expenditures attributable to periodontal disease in the United States are estimated at over $154 billion annually when periodontal treatment, tooth extractions, denture fabrication, and implant rehabilitation are considered together.[36,48] Dental implants, which have become the standard of care for replacement of periodontally lost teeth, especially among younger adults, cost between $3,000 and $6,000 per tooth and are largely excluded from most dental insurance plans, imposing substantial out-of-pocket burden on patients while representing a meaningful cost to Medicaid and other public payers in states that cover implant services.[33,36] Full-arch implant rehabilitation, required for the edentulous patient seeking functional tooth replacement, can exceed $40,000 per arch. Conventional complete dentures, while less expensive, are associated with accelerated alveolar ridge resorption, nutritional compromise due to reduced masticatory efficiency, and substantial psychosocial morbidity. Such costs are diffuse, difficult to capture in direct expenditure analyses, and nonetheless real.[34,35]

The indirect economic costs of periodontitis and edentulism through lost workdays, reduced labor productivity, social withdrawal, and depression associated with tooth loss add a further economic dimension that strengthens the case for prevention.[35,49] Tooth loss is consistently associated with reduced quality of life, impaired social functioning, and elevated rates of depression and anxiety in both community and clinical samples, and these psychosocial sequelae carry economic costs through reduced labor force participation and increased mental health service utilization that are not captured in dental expenditure data alone.[35]

Against this backdrop of direct, indirect, and systemic costs, the investment required to prevent periodontitis through early dietary intervention is modest: the USDA estimates the

incremental cost of meeting current whole grain standards in school lunch programs at approximately $0.10–$0.15 per meal; moving toward more intact, texturally challenging grain products would impose a similarly small marginal cost given that minimally processed whole grains are not systematically more expensive than refined grain commodities when purchased at institutional scale.[44,45] Dietary counseling integrated into existing pediatric dental visits requires no additional infrastructure and minimal additional training; the primary barrier is the absence of reimbursement incentives for such counseling in current dental benefit structures. Dental benefit reform that extends coverage for dietary counseling codes within the periodontal prevention context and is modeled on the expanded preventive counseling benefits already present in medical insurance for obesity and diabetes prevention would create the financial incentive necessary to incorporate this dimension of care into routine pediatric dental practice.[36,37]

The economic case for prevention is ultimately a case for temporal reallocation of healthcare investment: spending modestly earlier, during childhood and adolescence when the oral microbiome and gingival immune architecture are most amenable to dietary modification, to avoid spending substantially later for costs related to the surgical, prosthetic, and systemic disease management costs generated by uncontrolled adult periodontitis. This dynamic is well established in the preventive medicine literature for conditions including cardiovascular disease, type 2 diabetes, and obesity, and the bidirectional relationships between these conditions and periodontal disease suggest that periodontal prevention may generate returns on investment that substantially exceed those captured in dental expenditure analyses alone.[30,31,48] The present thesis, by identifying a mechanistically grounded and modifiable dietary determinant of periodontal homeostasis that is amenable to both individual clinical guidance and population-level policy

intervention, provides a scientific foundation for making this investment with greater precision and confidence.

**Concluding Remarks**

This thesis provides the first systematic experimental dissociation of the contributions of dietary texture and fiber content to periodontal host–microbe homeostasis across multiple integrated outcome domains. The convergent findings across biofilm biology, host immune phenotypes, and oral microbial community structure establish dietary texture as a biologically plausible, mechanistically grounded, and experimentally supported determinant of periodontal health—one that operates through distinct pathways from those mediated by fiber content alone. Grain refinement, by simultaneously removing fibrous bran encapsulation and reducing masticatory challenge, appears to create conditions dually permissive for oral dysbiosis and periodontal breakdown.

The implications of these findings extend from the bench to the bedside to the policy environment. Periodontitis is irreversible, prevalent, and costly, yet it is also preventable. Its prevention demands early intervention, beginning in childhood, when the oral microbiome is most ecologically plastic and when dietary patterns are most amenable to modification. The dental profession has historically underemphasized dietary texture as a clinical target for periodontal prevention, and the policy environments that govern what children eat have not yet fully integrated oral health as a rationale for whole grain and dietary texture standards. The present thesis contributes mechanistic evidence that should help close both gaps.[36,37,40,41]

Restoring the structural integrity of dietary fiber through the consumption of intact whole grain foods—and ensuring that institutional food environments make such foods accessible to all children regardless of socioeconomic status—may represent one of the most scalable, cost-

effective, and sustainable levers available for reducing the global burden of periodontal disease and edentulism. As periodontal disease continues to impose substantial costs on individuals, healthcare systems, and economies, and as its bidirectional relationships with cardiovascular disease, diabetes, and other systemic conditions become increasingly recognized,[30,31,48] the identification of modifiable dietary determinants of periodontal resilience becomes a matter not only of oral health but of whole-person and whole-population health. The present thesis contributes to this effort by establishing food texture as a mechanistically distinct, clinically relevant, and policy-actionable dimension of dietary influence on the periodontium.

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