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Microbiome, alveolar bone, and metabolites: Connecting the dots

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The oral microbiome (OM) is a diverse and dynamic collection of species, separated from alveolar bone by the oral mucosa. Pathogenic shifts in the OM (dysbiosis) during periodontitis are associated with an inflammatory response in the oral mucosa that drives alveolar bone resorption. Alveolar bone is also affected by metabolic disorders such as osteoporosis. Accumulating evidence has linked another microbial community, the gut microbiome (GM), to systemic bone metabolism and osteoporosis. Underlying this connection is the biologic activity of metabolites, byproducts of host and bacterial activity. Limited evidence also suggests that metabolites in the oral cavity signal between the OM and immune system, influencing both alveolar bone homeostasis and pathologic bone destruction in periodontitis. While the oral cavity and gut are connected through the gastrointestinal tract, dissimilar roles for known metabolites between these two niches exemplify the difficulty in translating knowledge on gut-derived metabolites and bone metabolism to alveolar bone. Integrated metabolomic, transcriptomic, and metagenomic approaches hold promise for resolving these challenges and identifying novel metabolites which impact alveolar bone health. Further interrogation through mechanistic testing in pre-clinical models and carefully controlled clinical studies have potential to lead toward translation of these discoveries into meaningful therapies.

KEYWORDS
alveolar bone, oral microbiome, gut microbiome, metabolites, periodontitis, osteoporosis

Introduction

The human body is colonized by trillions of microbes (1). Recent advances, including the Human Microbiome Project and the development of next generation sequencing technologies, have convincingly demonstrated that distinct microbial communities colonize different body sites and interact with host cells to modulate health and disease (2, 3). It is further established that maintenance of health requires a state of homeostasis between the microbiome and immune system across different body sites, also known as niches (4, 5). Two distinct niches, the gut and oral cavity, are characterized by a complex relationship between the host and gut microbiome (GM) and oral microbiome (OM), respectively (6, 7). Disturbances in these homeostatic interactions drive dysbiosis and inflammation and are associated with

several chronic diseases, including inflammatory bowel disease (IBD), type 2 diabetes (T2D), obesity, metabolic syndrome, osteoporosis, rheumatoid arthritis, Alzheimer's disease, periodontal disease, dental caries, and various cancers (8, 9). The role of the OM in driving alveolar bone destruction is well established (10), and a role for the GM in regulating systemic bone health has become increasingly appreciated (11, 12). Accordingly, the nature of the microbial-host interrelationships that regulate bone metabolism in health and disease are active areas of investigation.

Alveolar bone is the specialized portion of the mandible and maxilla which houses, supports, and protects the root structures of teeth (13). Formation and remodeling of alveolar bone is shaped by local factors, such as the eruption of teeth into the oral cavity and ongoing masticatory forces, and systemic regulation through hormonal and metabolic signaling (14, 15). Distinct from other skeletal structures, alveolar bone lies in close proximity to OM biofilms and undergoes resorption during the course of periodontitis, a chronic and widespread disease (16). The periodontitis-associated OM is characterized by dysbiotic biofilms on tooth and root surfaces containing several pathogenic species such as *P. gingivalis, Treponema denticola, Tannerella forsythia*, and *A. actinomycetemcomitans* (17). Concurrently, a heavy immune cell infiltration is present in the gingiva, the oral mucosal tissue surrounding the teeth, which drives osteoclast activity in the underlying alveolar bone (18, 19). Diseases that affect bone metabolism, such as osteoporosis, also affect alveolar bone health (20). Thus, an interplay between OM, the associated immune response, and local and systemic factors affecting bone shape the pathogenesis of alveolar bone loss.

Metabolites, the currency of bacterial-host crosstalk

Metabolites are the byproducts of microbial or host metabolism specific to the environment, modulating health by signaling to host cells and influencing bacterial community interactions (21). Host amino acids (22) and byproducts from glucose-related pathways (e.g., glycolysis and gluconeogenesis) (23) and mitochondrial metabolism (e.g., tricarboxylic acid cycle metabolites succinate, fumarate, and aconitate) (24) have well known roles in signaling within and between immune and bone cell populations. Microbial metabolite production, best characterized in the gut environment, is heavily driven by dietary intake, with fermentation of complex carbohydrates and proteins leading to production of short- and branched-chain fatty acids, and metabolism of proteins and peptides producing amines, phenols, and indoles from amino acids (25, 26).

Interactions between microbially-derived metabolites and host cells are increasingly recognized as drivers of human health and disease (21, 27). Extensive research in the gut has identified roles for microbially derived metabolites, including secondary bile acids, short-chain fatty acids (SCFAs), trimethylamine-N-oxide (TMAO), polysaccharide A, 4-ethyl phenyl sulfate, and catecholamines, in systemic diseases affecting bone (28–31). In contrast, there is currently a narrower understanding of the scope and nature of OM-derived metabolites and their role in alveolar bone health (**Figure 1**).

The emerging field of metabolomics has enabled cataloging of both well-known and novel metabolites using an array of platforms and techniques (32). The number of metabolite entries in the Human Metabolome Database, the most comprehensive collection of human metabolites, has burgeoned from 2,180 entries in 2007 to 217,920 annotated metabolite entries and 1,581,537 unannotated entries (33). These technologic advances in unbiased metabolomics have significant potential to (1) uncover the net biological activity in the oral cavity, (2) expand our knowledge of the pathogenesis of alveolar bone destruction beyond identifying specific bacterial species, and (3) identify novel targets for disease diagnosis, prognosis, and treatment.

Lessons learned from the gut microbiome

The microbiome colonizing the human intestine, known as the gut microbiome (GM), is the largest microbial niche in the human body and comprises a complex ecosystem with established roles in human health and disease (34). Initially formed *in utero* or at birth, the GM rapidly develops between ages 1–4 and continues to evolve in response to intrinsic and environmental factors such as geographic location, gender, diet, and antibiotic use (35). The intestinal mucosal epithelium serves as the interface between host and the microbiome, controlling interactions through the coordinated activities of mucus, epithelial cell junctions, immunoglobulin A, antimicrobial peptides, and immune cells (36, 37). Nutrients and metabolites also pass through this barrier to interact with local cells or enter the circulation (38).

Bone remodeling and homeostasis are regulated by a network of systemic hormones, including parathyroid hormone (PTH), calcitonin, FGF23, 1,25-dihydroxyvitamin D_3 (Vitamin D), and estrogen. The GM is considered an endocrine organ (39) and animal models show that altering or preventing GM development influences skeletal bone mass and osteoclast activity (40–42). Gut microbes synthesize vitamin K_2 which stimulates osteoblast activity and is a cofactor for post-translational modification of osteocalcin (12). Disruption of the ecosystem with antibiotics inhibits Vitamin K_2 synthesis and reduces bone quality (43). Enzymes secreted by gut microbiota can metabolize or re-activate estrogen, altering circulating or excreted levels (44). GM dysbiosis can also mediate estrogen deficiency-related bone loss through

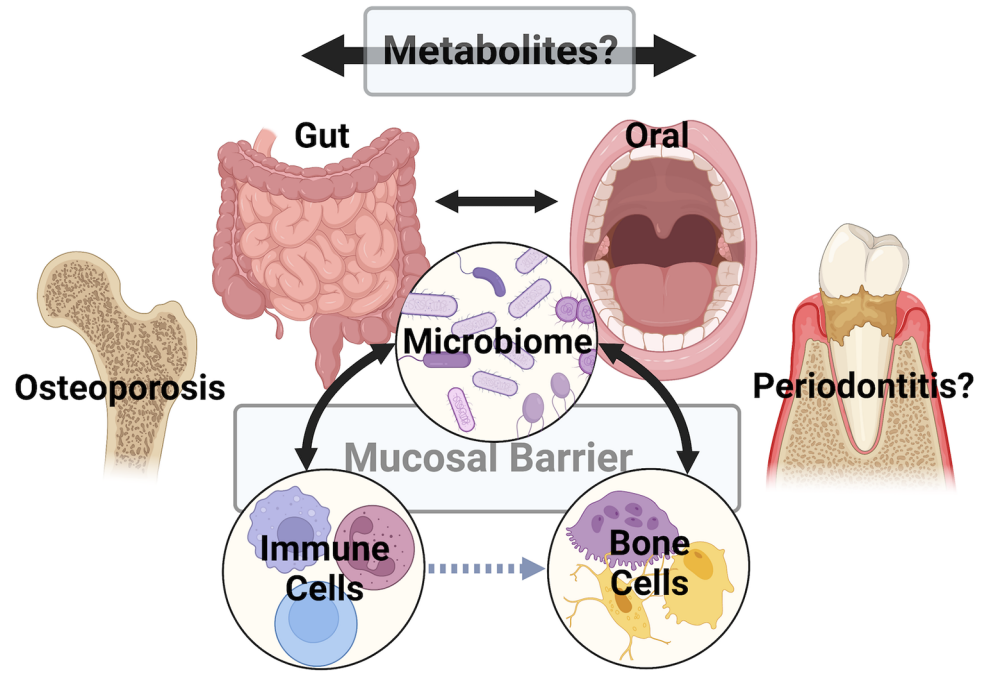

FIGURE 1
A multitude of studies have explored the connections between the gut and/or oral microbiomes, the host immune system, and bone cells (i.e., osteoblasts, osteocytes, and osteoclasts). Recent work suggests that metabolites are key signaling factors in these pathways (represented by black bidirectional arrows), acting directly or indirectly (i.e. *via* the immune system – gray dashed arrow) to influence pathologic bone disorders like osteoporosis. A significant challenge is translating knowledge gained from studies of the gut and osteoporosis to the oral cavity to understand if metabolites play similar or distinct roles in the metabolism of alveolar bone. Underlying this challenge are differences in mucosal barrier structures, microbiome populations, and immune cells between the gut and oral mucosa. Figure created with BioRender.com.

increases in mucosal permeability, immune cell numbers, and inflammatory cytokines (45, 46).

The GM can enable serotonin production by enterochromaffin cells (47) and deconjugate bile acid compounds and further metabolize them to secondary bile acids such as lithocholic and deoxycholic acid (48). Gut-derived serotonin may inhibit bone formation (49) and lithocholic acid can bind vitamin D receptor (VDR), leading to inactivation of vitamin D and decreased intestinal calcium absorption (50). Bile acids can also signal enteroendocrine cells to release GLP-1 which promotes bone formation and inhibits bone resorption (51). Hydrogen sulfide (H_2S) is produced by gastrointestinal cells and the GM (52). Loss of H_2S results in osteopenia in mice (53) and administration of an H_2S donating compound in ovariectomy-treated mice improves bone formation (54).

Emerging evidence points to gut-derived short-chain fatty acids (SCFAs) as modulators of systemic health and bone maintenance [see reviews (11, 12, 55, 56)]. In brief, SCFAs, including butyrate, propionate, and acetate, are primarily produced by microbial fermentation of non-digestible polysaccharides and are rapidly absorbed through intestinal mucosa, acting as a source of energy for both host and microbiota (56). While SCFAs can directly suppress osteoclast activity and promote osteoblast differentiation (57, 58), signaling between SCFA and endocrine organs or immune cells may underly the connection between GM and bone. Gut microbial colonization or SCFA supplementation is associated with the production of insulin-like growth factor 1 (IFG-1), an important hormone for skeletal growth and bone mass maintenance (59). SCFAs, including butyrate, promote proliferation and differentiation of regulatory T cells (Treg) (60) which may reduce bone absorption by interfering with osteoclast development and activity (61). Butyrate can increase Treg numbers in the intestine and bone marrow which signals to CD8+ T cells to produce WNT10b, a bone anabolic signaling factor (62). Butyrate produced by GM may also regulate PTH-mediated bone formation through signaling in dendritic cells and Tregs (63).

Probiotics have been widely studied as a means to target osteoporosis *via* manipulation of the GM (64). A clinical study showed *Lactobaciillus reuteri* probiotics increased BMD and elevated butyrylcarnitine, which can act as pool and transporter of butyrate (65). Prebiotics are non-digestible oligosaccharides that are selectively fermented in the colon and support growth of specific bacterial species. Positive results for prebiotics in animal models, including increased calcium absorption and improved BMD and bone strength,

have been primarily attributed to fermentation of prebiotics to SCFAs by GM (66). Clinical trials have further indicated that prebiotics can increase intestinal calcium absorption (67).

Altogether, the GM plays a critical role in regulating systemic bone metabolism, in part, through production of metabolites. GM-derived metabolites act both locally and systemically on host cells to drive immune responses that shape bone metabolism. Improved understanding of GM metabolites and their role in shaping bone health have led to development of therapeutic interventions, including probiotics and prebiotics, suggesting that probing the connection between the oral cavity and gut and identifying similar pathways in the oral cavity has promise for improving alveolar bone health.

The oral-gut-bone connection

Ingested saliva, food, and drink directly connect the OM and GM (68, 69). Patients with conditions characterized by GM inflammation and dysbiosis, such as inflammatory bowel disease, have an altered OM, increased numbers of OM-derived species in the gut, and higher rates of periodontitis (70, 71). Studies in mice suggest that ingested OM bacteria can reach the gut and induce an inflammatory immune response (72), and immune cells exposed to OM can reach the gut to interact with OM-derived gut microbes (73). Gut colonization with specific bacterial species can also influence T cell development in alveolar bone marrow and increase alveolar bone osteoclast activity, further illustrating the potential bidirectional mechanisms whereby microbial populations in both gut and oral cavity can help disrupt or maintain bone homeostasis (74).

Studies probing the oral-gut connection have shown that adminstering oral *P. gingivalis* modifies the GM and alters serum and gut metabolite profiles (75, 76), including increasing gut lactic acid and reducing succinic acid and butyrate levels (77). Additional evidence connecting GM, metabolites, and alveolar bone has been provided by animal studies of probiotic administration or diet alterations. In ovariectomized rats, probiotics increased levels of butyrate-producing GM and reduced osteoclast and Th17 cell numbers while increasing Treg cells and minimizing maxillary bone loss during ligature-induced periodontitis (78). Transplantation of fecal contents from high fat diet (HFD) obese mice altered host GM and gut and serum metabolite compositions with little change in the OM while increasing Th17 cells in submandibular and mesenteric lymph nodes and aggravating alveolar bone loss in experimental periodontitis (79). One metabolite of purine degradation, uric acid, was increased in serum with HFD fecal transplant and induction of periodontitis, and administration of allopurinol suppressed alveolar bone destruction in uremic mice (79).

Overall, these findings lend support to the concept that the oral health is connected to systemic health and highlight distinct molecular pathways connecting the gut and oral microbiomes and the immune system through metabolites. Whether such mechanisms identified in mouse models can be translated to meaningful interventions in humans is still unknown. Nevertheless, such studies provide further motivation for studying the role of metabolites in bone health and, in particular, within the oral niche.

Oral metabolites and alveolar bone

The oral cavity is rich in byproducts of host and OM metabolism (80). Saliva and gingival crevicular fluid (GCF) show distinct profiles of metabolite compositions between health and periodontitis (81–83) with clinical studies showing specific associations between periodontitis and increased levels of arachidonic acid, purine, pyrimidine, glutathione, and amino acid metabolites (84–87). Accordingly, various metabolites have been explored as predictors of gingival inflammation or periodontitis (88) or as factors that regulate the disruption or maintenance of the gingival epithelial barrier (junctional epithelium) (89). However, clear evidence is lacking for how specific metabolites or metabolic pathways act to help maintain alveolar bone in oral health or aggravate bone destruction during periodontitis.

Existing studies on oral metabolites and alveolar bone have focused on butyrate, and contrary to the gut, have ascribed it a pathogenic role in periodontitis (**Figure 2**). This distinction may be due to several factors, including differences in butyrate concentrations, mucosal tissue structure, and microbial populations between GM and OM environments (90, 91). Periodontitis-associated oral bacteria, *P. gingivalis* and *F. nucleatum*, produce butyrate (92). Further, butyrate can stimulate heme production which supports growth of periodontal pathogens like *P. gingivalis* (90). Butyrate concentrations in periodontal pockets can reach up to 14 mM (93) with levels correlating to periodontal disease severity (94) and decreasing in GCF after periodontal treatment (95). While butyrate levels may be similar or higher in the colon compared to the oral cavity, a much lower concentration may actually reach colonic epithelial cells after penetrating through the thick colon mucous layer (96). A recent animal study found that butyrate could disrupt the periodontal junctional epithelial barrier (97). This finding, coupled with *in vitro* studies showing a negative effect of butyrate on different oral cell types (90, 91), and in particular, epithelial cells (98), suggests that differences in the mucosal barrier anatomy between gut and periodontal tissues could account for some of the opposing effects of butyrate on alveolar vs. other bone sites.

Conceivably, OM-derived butyrate and other SCFAs signal to immune, epithelial, and stromal cells in periodontal tissues

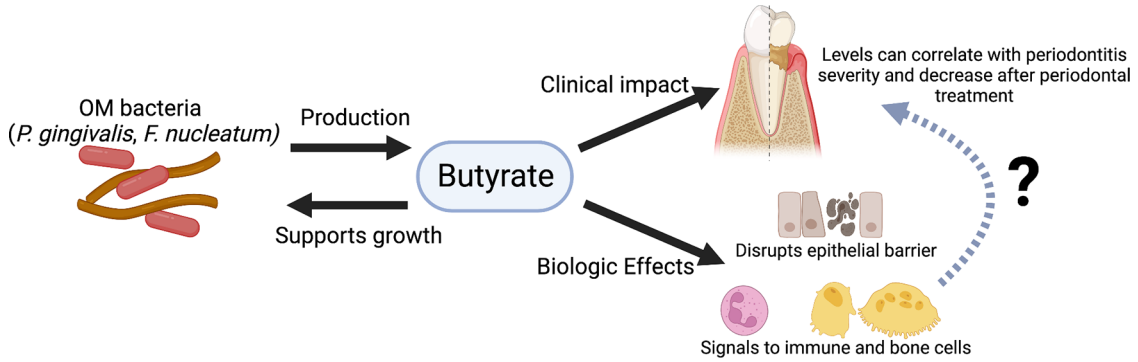

FIGURE 2
Graphical summary of evidence for butyrate's role in periodontitis. Periodontitis-associated bacteria found within the OM (e.g., *P. gingivalis* and *F. nucleatum*) produce butyrate, which in turn can support their growth. Clinical studies have shown an association between butyrate levels and periodontitis and found that butyrate levels decrease after periodontal treatment. Experimental studies indicate butyrate may disrupt the junctional epithelial barrier and can signal to immune and bone cells. However, the exact mechanisms connecting butyrate and its possible biologic effects to periodontitis and alveolar bone are still unknown. Figure created with BioRender.com.

which could then interact with osteoblasts and osteoclasts. SCFAs appear to affect the ability of neutrophils to respond to the periodontal pathogen *A. actinomycetemcomitans* (99). Mice deficient in the SCFA receptor FFAR2 showed increased alveolar bone loss and decreased maxillary bone density, with the latter partially rescued by a high fiber diet (100). While osteoclasts derived from FFAR2-deficient mice showed increased *in vitro* differentiation, the only SCFA which could inhibit this activity was butyrate, indicating that butyrate acted independently of the FFAR2 receptor.

Clearly, further work is needed to identify how metabolites beyond SCFAs affect alveolar bone and to better understand how butyrate and other metabolites modulate alveolar bone metabolism through the oral mucosal immune response to OM biofilms. Additional questions inspired by the role of GM metabolites in bone health may provide insight. Do metabolites produced in the oral cavity act on the oral mucosal immune system similar to how the GM indirectly influences bone health? Do differences or similarities between the oral and gut niches underly the impact of oral metabolites on alveolar bone? Answers to these and other questions, aided by advances in scientific techniques, may provide new options for diagnosing, treating, or preventing periodontitis and the associated loss of alveolar bone.

The path forward

The bulk of studies on periodontitis and alveolar bone thus far continue to focus on OM characterization through either 16S or whole genome shotgun sequencing approaches and interrogating the host immune response. Work investigating the biologically active small compounds that determine the net functional activity in the oral environment remains scarce. However, such investigations are beginning to emerge, enabled by technological advances in metabolomics. Indeed, recent studies have demonstrated that combining metabolomics with transcriptomics, 16S DNA genomics, and other unbiased techniques has potential for identifying new molecular pathways and therapeutic targets for periodontitis and alveolar bone loss (101–103).

In parallel, rigorous studies are required for determining the mechanisms behind oral metabolites and alveolar bone. The majority of existing studies on oral metabolites utilize *in vitro* models of homogeneous cells and/or bacterial populations. Such approaches have significant limitations in their ability to recapitulate the complex environment of subgingival biofilms, oral mucosal tissues, and underlying alveolar bone. Thus, carefully controlled animal studies should be designed to investigate the mechanisms behind host and bacterial metabolites and alveolar bone health.

The translation of findings on known or novel oral metabolites to effective therapies for maintaining alveolar bone face specific challenges in study design and analysis. Characterization and validation of possible targets for therapy will entail clinical studies with rigorous study design, careful cohort stratification, and inclusion and exclusion criteria to ensure application and reproducibility. Data integration and analysis with multi-omics approaches is challenging due to heterogeneity in the data format from each -omics technologies, discrepancies in annotation, and non-uniform missing data from different data. Additionally, the computational complexity and lack of standardization for analytical and bioinformatic pipelines may hinder reproducibility across studies. Thus, the introduction of standardized protocols for clinical studies and computational approaches, along with techniques to accommodate for data heterogeneity and missing data, are critical for the success of future work. With these tools in hand, an integrative multi-

omics approach combining metabolomics, metagenomics, transcriptomics, and other -omics techniques may be able to resolve the interconnected roles of the OM and immune response in alveolar bone health and disease.

Data availability statement

The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding author.

Author contributions

DF and SMG: conceptualization, writing – original draft, writing – review and editing. SMG: funding acquisition. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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