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Host-microbe interactions: Profiles in the transcriptome, the proteome, and the metabolome

Trang Nguyen[1], **Lea Sedghi**[2], **Sean Ganther**[2], **Erin Malone**[2], **Pachiyappan Kamarajan**[2], **Yvonne L. Kapila**[2]

[1]School of Dentistry, University of California San Francisco, San Francisco, California, USA

[2]Department of Orofacial Sciences, School of Dentistry, University of California San Francisco, San Francisco, California, USA

1 | INTRODUCTION

An appreciation for the complexity of host-microbe interactions has evolved from a single organism etiology (Koch's postulates) to an understanding of the intricate polymicrobial interactions and community dysbiosis involved in disease processes.[1,2] Metagenomic studies of the oral microbiome provide a greater understanding of microbial ecology in different states of health and disease. The application of functional genomic technologies ("-omic" technologies) and high-throughput analyses, including metagenomics, transcriptomics, proteomics, and metabolomics, to the oral cavity has given a new perspective to the underlying mechanisms of periodontal disease pathogenesis and progression.[3,4] These analyses reveal variations in the composition of the microbial community in states of health and disease, as well as how variations in these populations influence the host response and the subsequent phenotypic presentation of disease.[5] Periodontal disease is largely driven by states of microbial dysbiosis and is exacerbated by an acute host response to such conditions.[6,7] Linking the processes that underlie the host-microbe interactions in periodontal disease is critical to understanding this interface. Furthermore, analyses relating to changes in host cell transcriptional profiles, protein function, and metabolic responses contribute to a broader understanding of the intricate and multifaceted responses at the molecular level. Integration of such analyses confers a comprehensive assessment of the impact of microbial communities on the host cell response. Research using transcriptomics, proteomics, and metabolomics to study periodontal disease has shed new light on disease pathogenesis and host-microbe interactions, processes, and pathways.

2 | TRANSCRIPTOMICS

Transcriptomics, or meta-transcriptomics, has helped to elucidate the role of various RNA subtypes in periodontal health and disease. The main methods currently used in transcriptomic studies include RNA sequencing and microarray analyses[3,8–19] (Table 1). RNA sequencing effectively demonstrates periodontitis-related gene expression.[12]

*Correspondence: Yvonne L. Kapila, Division of Periodontology, Department of Orofacial Sciences, School of Dentistry, University of California San Francisco, 513 Parnassus Avenue, S616C, Box 0422 San Francisco, CA 94143, USA. Yvonne.Kapila@ucsf.edu.

Periodontal and gingival tissues are the predominant sources for samples used in transcriptomic analyses of periodontitis. Neutrophils have recently been explored as a potential source in transcriptomic studies of chronic periodontitis, as neutrophils release disease-related products, such as enzymes that cause cell membrane damage and apoptosis. Additionally, neutrophil recruitment increases during chronic infection in the oral cavity, and neutrophils change their gene expression profiles as they migrate from the central circulation to the oral cavity in patients with chronic periodontitis.[18] Human fibroblasts have also been analyzed in tran scriptomic studies of periodontitis, providing a more comprehensive overview of periodontitis-related fibroblast transcriptomes.[11]

2.1 | Host transcriptomics

Transcriptomic studies of periodontal and gingival tissues have revealed a variety of important transcripts involved in the process of periodontal disease pathogenesis. In healthy tissues from patients with a history of generalized aggressive or chronic periodontitis as compared to no history of periodontitis, a significant increase in the expression of genes related to the immune response and natural killer cell receptors (natural killer cell immunoglobulin-like receptor DL4, interleukin-6, and selectin E) was observed. By contrast, genes related to neural processes and proliferation/differentiation of keratinocytes were underexpressed in generalized aggressive periodontitis. However, their expression decreased in tissues affected by chronic periodontitis. Chronic periodontitis was characterized by increased expression of genes involved in responses to external stimuli, and underexpression of immune system-related genes.[10,12] The altered expression of these immune response genes suggests an important and differential role for the innate and adaptive immune response (B and T cells) in various states of periodontal disease.[12] A high level of B cell and plasmacyte gene expression was also found in a study examining periodontitis-related transcriptomes in human and nonhuman primates.[13,20] A recent study by Lundmark et al[20] found an upregulation of immunoglobulin lambda like polypeptide 1 and immunoglobulin lambda like polypeptide 1, genes involved in B cell development in gingival tissues from periodontitis sites. The diversity of B cell responses was further altered in the context of aging, revealing an age-related adaptive immune response in gingival tissues.[13] An upregulation of glycoprotein-producing genes, such as mucins, has also been identified in transcriptomic studies of periodontitis.[8] As the production of glycoproteins is involved in many of the activities of neutrophils and inflammatory mediators, the increased expression of these genes might contribute to innate immune responses present in periodontitis.[8]

Aging also causes changes in bone biology-related gingival transcriptomes. Specifically, genes related to osteoclast function exhibit enhanced expression (secreted phosphoprotein 1, toll like-receptor 4, matrix metallopeptidase 8, and transcription factor EC), and those related to osteoblast activity are impaired (protein phosphatase 4 regulatory subunit 3C and SMAD family member 5).[14] Tissues associated with periodontitis exhibited genes involved with a local inflammatory response driving bone and connective tissue destruction (FOS protooncogene, AP-1 transcription factor subunit, interleukin-6, toll like receptor 4, matrix metallopeptidase 9, matrix metallopeptidase 10, and secreted phosphoprotein 1). As noted earlier, increased expression of genes associated with antigen-dependent activation, B cell

activation, B cell proliferation, and B cell differentiation/maturation were present in periodontitis in adults and aged animals.[13]

The effects of smoking on the periodontium have been widely investigated in clinical and animal studies.[21–23] Transcriptomic studies have revealed that smoking mediates epigenetic modifications on the extracellular matrix organization of the periodontium, which subsequently enhances disease progression and accelerates tissue destruction.[9]

2.2 | Microbial transcriptomics

The transcriptome of the oral microbiome has also been investigated.[16,17] Metagenomic and metatranscriptomic analyses are two general approaches that have been used. These combined approaches have been applied to periodontal research and have helped to define the complexity of the supra- and subgingival microbiome.[17] A main target of periodontitis-related microbial transcriptomic research is mRNA expression. Small RNAs have also been investigated and are similarly altered/upregulated in the context of periodontitis. Small RNAs, in general, regulate the translation of mRNA by attaching or detaching to the ribosome-binding sites; when there is an increase in mRNA, the amount of small RNAs increases correspondingly.[17] It has been extensively documented in periodontal-related literature that the composition of the oral microbiota changes in the transition from health to disease; however, differences in community composition vary across individual patients. Despite the variability that exists among individuals, transcriptomic studies of the oral microbiome have shown that health- and disease-associated communities have defined differences in metabolism that are conserved between patients. However, the metabolic gene expression of individual species is highly variable between patients. Thus, disease-associated communities exhibit conserved metabolic profiles that are mediated by a patient-specific group of microbes.[16]

Periodontal disease is not only associated with changes in human and bacterial transcriptomes, but also with that of viral or bacteriophage communities. Significant differences exist in the gene expression of viruses (or the virome) between healthy individuals and those with periodontitis. RNA sequencing reads of siphoviruses (that infect Firmicutes) were significant to both periodontal health and disease. However, genes from lytic phages were highly expressed in saliva from individuals with periodontal disease.[15] Lytic phages were also significant in subgingival biofilms of individuals with periodontal disease.[24]

Although current research on periodontitis and the associated transcriptome is limited, recent studies have shown promising results that may be beneficial to the diagnosis of periodontal disease via the development of gene biomarkers. In addition, these approaches may be beneficial in the context of peri-implantitis, as the transcriptome of tissues from patients with peri-implantitis was significantly different from the transcriptome of tissues from patients with periodontitis.[19] Thus, transcriptomic analyses in peri-implantitis may be a potentially new area of research.

3 | PROTEOMICS

Proteomics is a science that refers to the systematic and large-scale analysis of the entire proteome or protein content of a cell, organism, or system to understand better the protein signature of biologic and pathologic states. Many technological tools have been applied in proteomic science, including protein purification, mass spectrometry, surface-enhanced laser desorption/ionization mass spectrometry, polyacrylamide gel electrophoresis, high performance liquid chromatography, and matrix-assisted laser desorption ionization, to measure protein profiles in biomedical sciences.[25] The development of mass spectrometry, novel computational methods, proteomics software, advanced machine learning tools, data management systems, and data repositories has produced many advances for large-scale protein analysis.[26] Many proteomic studies have been carried out using mass spectrometry-based technology at a qualitative level[27] with "label-based" proteomics. "Label-based" proteomics requires the use of expensive stable isotope reagents. Further advancements were introduced for direct large-scale technology using a "label-free" approach, in which fewer steps are involved in labeling, although this can limit the number of identified and quantified proteins. There are challenges and limitations from analyzing proteomic samples using mass spectrometry-based label-free quantitation methods.[28] These combined approaches have significantly enhanced proteomic analyses, which have been used to understand disease processes, including periodontal disease.

3.1 | Proteomic human studies

Many studies have been carried out in humans with the goal of discovering a proteomic signature for periodontal health[29,30] (Table 2). In addition, the proteome of periodontally diseased conditions has been actively investigated in human samples.[31–49] The investigations targeted many forms of the disease, ranging from gingivitis[44] to mild, moderate, chronic,[33,36,37,41,47] and aggressive periodontitis.[42,46,48] Most studies have focused on analyses of samples from saliva,[30,34,36,38,44,47,49] gingival crevicular fluid,[29,31,33,37,41,43,45,48] and sulcular/gingival tissues.[35,40]

Samples of saliva and gingival crevicular fluid can be collected noninvasively. However, the technique for gingival crevicular fluid sampling is more sensitive[50] and only a limited sample volume can be effectively collected in comparison with the volume of saliva collected. Both sample types exhibit a dynamic range of protein abundances. In contrast to gingival crevicular fluid, which comprises a small percentage of the total protein content, saliva contains the vast majority of proteins, mostly intracellularly glycosylated proteins originating from the major maxillofacial salivary glands.[51,52] Whole saliva is beneficial in early patient screening and large-scale population sampling.

Because of its site-specific nature, gingival crevicular fluid is useful for analyzing different sites within the same patient and may contain specific periodontal disease-related biomarkers. However, as a result of the limited sample volume,[50] gingival crevicular fluid presents challenges for subsequent processing and analysis, further complicated by the high abundance of albumin in these samples.[53–58] Recent studies have applied an albumin-depletion method involving trichloroacetic acid/acetone precipitation as a new strategy to decrease the albumin signal.[33] Another limitation of gingival crevicular fluid, unlike saliva,

[59] is that its volume increases with the severity/progression of periodontal disease and this is not age-dependent.[60] However, given its stability and specificity, recent studies have pooled gingival crevicular fluid as a potential alternative to saliva in proteomic analyses.[55] One additional challenge with gingival crevicular fluid is that proteomic analyses which use mass spectrometry cannot detect cytokines,[37,61] as their concentrations are very low in gingival crevicular fluid samples.[27,48]

Periodontal sulcular/gingival tissue has recently been used for proteomic analyses as it is molecularly accessible and contains significant levels of periodontitis-related proteins.[35] Compared with conventional detection methods involving gel electrophoresis, the recent use of liquid chromatography combined with mass spectrometry has revealed a large number of proteins associated with periodontitis lesions.[54] In addition to human proteins, bacterial proteomes have also been identified in oral fluids, suggesting the presence of planktonic forms of oral microbes within these biofluids.[34]

In terms of the human oral proteome, apoliporoteins, immunoglobulins, and other components of the immune response, cytoskeletal proteins, and neutrophil-derived extracellular histones have been found in gingival crevicular fluid in the context of periodontitis.[37,39–41] In chronic periodontitis, many potential biomarkers of periodontal disease have been identified via proteomic studies,[50] including histones, cytoskeletal-related proteins, extracellular matrix proteins, and antimicrobial proteins. Histones are nuclear DNA-binding proteins that play a role in multiple biologic processes, including organization of the DNA structure. Histones, including H2A and H2B, which function in response to inflammation, and H4, which functions in cell organization, are not usually present in periodontally healthy patients. However, they are present in larger amounts in patients with gingivitis and chronic periodontitis.[27,48,61] Apart from histones, cytoskeletal-related proteins, including actins and keratins, are also present in periodontitis, and reflect epithelial turnover caused by cell destruction from bacterial invasion.[62] In addition, actins are related to osteoclast activity, and an increase in actin levels might indicate an increase in bone loss.[62] Other proteins, including extracellular matrix and antimicrobial proteins, are also found in the context of chronic periodontitis. Glycoproteins, such as fibronectin, are secreted at higher levels in the extracellular matrix during the progression of periodontitis.[63,64] Also, protease inhibitors, such as cystatins, are found in the early stages of periodontal inflammation.[48] Furthermore, protein secretion varies before and after mechanical treatment of periodontitis. After standard mechanical periodontal treatment, inflammatory mediators are altered and, hence, the proteins related to this process predominate.[49] Aggressive periodontitis is characterized by neutrophil-secreted proteins, which function as inflammatory mediators and modulators of biofilm formation.[46] Wound-healing proteins related to cell migration, adhesion, and proliferation have also been associated with aggressive periodontitis.[46] In general, proteomic studies seek to discover biomarkers that may be useful in periodontal disease diagnosis, prognosis, and treatment to assist clinicians in disease management.

3.2 | Proteomic animal studies

Animal studies have also been carried out to discover a proteomic signature of periodontal disease[25,30,61] or of the response to the treatment of periodontitis.[30] Although the number of proteomic studies carried out in animals is limited, this type of study is useful for exploring novel therapies for periodontitis, which are challenging to carry out in humans. For example, proteomic-based animal studies, such as those conducted in primate and canine models, reported the effect of complement system-inhibiting proteins in the treatment of periodontitis in preclinical experimental periodontitis.[30] Proteomic-based animal studies may be beneficial in examining disease progression from healthy states to gingivitis and periodontitis.[65]

3.3 | Proteomic in vitro studies

Many in vitro proteomic studies related to periodontal disease and periodontal pathogens have been carried out in the last decade.[66–81] Of the oral pathogens examined,[81] the two most commonly investigated bacterial species are *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis*.[66,67,69,70,73,76–78,80] *Porphyromonas gingivalis*, a major pathogen in periodontitis, releases virulence factors, such as gingipains, through outer membrane vesicles[66] via a C-terminal domain signal system.[76] These outer membrane vesicles mediate multiple pathogenic functions, including biofilm formation. Outer membrane vesicles are also associated with bacterial survival and host immune response avoidance.[70] Proteomic studies recently revealed that outer membrane vesicle development depends on the heme conditions, which must be suitable for bacterial growth.[66] *Porphyromonas gingivalis* also possesses the ability to glycolyze host proteins for its growth and colonization by producing glycolytic enzymes at the outer membrane.[77]

One main benefit of performing in vitro proteomic studies is the ability to dissect out the host response to specific oral microbial pathogens or their proteins. For example, many types of bacteriocin, bacterially produced antimicrobial peptides, have been investigated in terms of their ability to inhibit bacterial colonization, and thereby modulate subsequent changes in pathogenesis.[69,82] In vitro proteomic studies also allow the examination of a more complex polymicrobial environment and inclusion of multiple species in the analysis.[79] Among the species explored in these complex biofilm environments, *P. gingivalis* and *Treponema denticola* have shown the most robust effects in biofilm communities. Proteomic studies have also shown that *A. actinomycetemcomitans*, a major pathogen in aggressive periodontitis, produces many outer membrane vesicles, similar to *P. gingivalis*. These outer membrane vesicles contain many effector proteins that are internalized into human host cells.[67,73]

4 | METABOLOMICS

Metabolomic approaches were developed more recently than metagenomics, transcriptomics, and proteomics. Metabolomic analysis is defined as the study of the complete set of metabolites produced within a living system and provides insight into the enzymatic pathways and intricate networks that are encoded within the genome. This analysis provides large-scale metabolite profiles of a cell, tissue, or fluid/sample from a

distinctive condition or state. Metabolomic analysis provides a metabolic perspective to functional genomics and, in the context of disease, delivers important information regarding changes in metabolic pathways via analysis of differential metabolite components in the diseased and healthy phenotypes.[83] Metabolomic studies offer many benefits over alternative functional genomic analyses, including the ability to attain a more realistic representation of the cell state,[76] the ability to connect various pathways via the observation of pathway intermediates,[84–86] and an unbiased evaluation of the cell state under specific conditions.[87,88]

Monitoring metabolite profiles in body fluids to understand disease etiology has been a common focus of this approach.[89] The identification of metabolites as potential biomarkers of periodontal disease status or pathogenesis is of interest for many reasons, including to understand the metabolic mechanisms underlying periodontal disease and to target patient treatment. Changes in metabolite composition associated with states of disease may provide the identification of metabolic biomarkers that can be used in a variety of applications, including early disease detection, evaluation of current disease status, and examination of pathways triggered or altered in the diseased state.[83,90] The most common approaches used for metabolomic studies include analyses of human body fluids by gas chromatography-mass spectrometry, liquid chromatography-mass spectrometry, and nuclear magnetic resonance spectrometry.[91]

Changes in metabolite composition reflect altered cellular/tissue function and phenotype, and are therefore important in understanding the microbial-host response in periodontal disease. Although the metabolomic approach contributes to understanding the complexity of the disease, the substantial information that metabolomics provides is limited by the difficulty in identifying and quantifying many intracellular and extracellular metabolites.[84,92] The challenges associated with metabolomic studies include the high variability that exists in the chemical structure and properties of the metabolites.[93] Nonetheless, metabolomic analyses offer the ability to understand the endpoint of complex pathways driving periodontal disease pathogenesis. The identification of key metabolites as biomarkers of periodontal disease has broadened the metabolic perspective of microbial-host interactions in periodontal disease pathogenesis.[94]

4.1 | Metabolomic sampling

Metabolic biomarkers of periodontal disease have been identified via the analysis of saliva, serum, plaque, and gingival cervical fluid (Table 3). Biomarkers of periodontal disease include metabolites associated with inflammation, oxidative stress, tissue degradation, and bacterial metabolism.[91] The use of various analytic methods and different patient sample types (gingival crevicular fluid, saliva, biofilm) in metabolomic analyses has enhanced the characterization of specific metabolic signatures associated with periodontal disease. In addition, incorporating different stages and types of periodontal disease and concomitant systemic diseases, as well as using site-specific analyses, has further enriched these studies. In this section we discuss recent efforts to advance the characterization of metabolic signatures associated with periodontal disease that reflect the collective host-microbial interactions within these tissues.

4.2 | Metabolomic human studies

Untargeted metabolomics has been used to distinguish the metabolic signature of individuals with periodontal disease from that of healthy individuals.[95,96] In addition, metabolic profiles have been used to discriminate between various stages and progression of periodontal disease. Nuclear magnetic resonance spectrometry-based metabolomics was recently used to characterize salivary phenotypes associated with both chronic periodontitis and generalized aggressive periodontitis compared with those of healthy individuals.[94] Significantly higher levels of proline, phenylalanine, and tyrosine, and significantly lower levels of pyruvate, *N*-acetyl groups, and lactate were identified for both the chronic periodontitis and the generalized aggressive periodontitis groups compared with healthy controls. However, unique metabolic profiles for the chronic periodontitis and generalized aggressive periodontitis groups could not be identified.

The metabolic profile of individuals with generalized aggressive periodontitis was further differentiated from that of healthy individuals by analyzing serum and gingival crevicular fluid using gas chromatography-mass spectrometry.[91] Metabolomic analysis of serum samples from patients with generalized aggressive periodontitis revealed decreased levels of 2-deoxyguanosine, glutathione, adipic acid, and 2,5-dihydroxybenzaldehyde, and increased levels of urea and alloinositol compared with samples from healthy individuals. Metabolomic analysis of gingival crevicular fluid revealed decreased levels of glutathione, 2-ketobutyric acid, glycine-d5, and thymidine, with elevated levels of noradrenaline, ribose, dehydroascorbic acid, lysine, and xanthine. Together these results indicate that there are increased metabolic markers of oxidative stress, purine degradation, tyrosine and pyrimidine metabolism, and bacterial biochemistry in generalized aggressive periodontitis.

The identification of biomarkers to measure the severity of periodontal inflammation and to identify disease-associated metabolic signatures of the periodontal microbiota was carried out using gas chromatography-mass spectrometry analysis of pre- and postdebridement saliva.[97] Following debridement, various salivary metabolites (4-aminobutyric acid, cadaverine, phenylalanine, 5-aminovaleric acid, succinic acid, putrescine, hydrocinnamate, ornithine, and fructose-6-phosphate) in the high inflammation group were reduced. The metabolites that were reduced following debridement in the low inflammation group included tryptophan, glutamine isoleucine, fucose, ethanolamine, and alanine. The high inflammation group was characterized by having increased polyamine metabolism, arginine and proline metabolism, butyric acid metabolism, and lysine degradation. Cadaverine and hydrocinnamate were identified as highly specific salivary markers for periodontal inflammation severity.

Distinct metabolic markers of periodontal disease were further identified using nuclear magnetic resonance spectroscopic analysis of salivary samples from patients with varying degrees and types of periodontal disease (mild, moderate, chronic, severe, and aggressive disease) vs healthy controls.[98] Patients with periodontitis had distinct metabolic signatures characterized by an increased concentration of butyrate, consistent with previous studies linking short chain fatty acid production to red- and orange-complex bacteria, including *P. gingivalis* and *Fusobacterium nucleatum*. Decreased concentrations of fucose, lactate, acetate, *N*-acetyl, gamma-aminobutyrate, 3-D-hydroxybutyrate, pyruvate, methanol,

threonine, and ethanol were observed in patients with periodontitis compared with healthy controls.

Metabolomic studies have also investigated the association between periodontal disease and chronic systemic disease. To identify key metabolites associated with gingivitis and periodontal disease and/or diabetes, liquid chromatography-mass spectrometry and gas chromatography-mass spectrometry were used to examine the metabolic profiles of plasma and saliva from patients with gingivitis or periodontal disease who had diabetes or were systemically healthy.[95] Patients with diabetes had decreased 1,5-anhydroglucitol and increased glucose in saliva, as well as increased serum alpha-hydroxybutyrate. The biochemical pathways that increased in patients with periodontal disease compared with healthy patients without diabetes included purine degradation (oxidative stress), dipeptides (macromolecular degradation of proteins), amino acid metabolites (p-cresol sulfate, bacterial), carbohydrates (monosaccharides indicative of amylase activity), energy metabolites (trichloroacetic acid cycle, indicative of energetic stress), uridine (DNA/RNA degradation), allantoin, omega-6 fatty acids (inflammation), fatty acids, and acetylcarnitine. Significant metabolites associated with purine degradation and antioxidant status that were increased in salivary samples from patients with diabetes included inositine, guanosine, and xanthine. Additionally, significant metabolites associated with fatty acids and sphingomyelins that were increased among patients with periodontal disease and diabetes included 12-hydroxyeicosatetraenoic acid, lineloate (18:2n6), lineolate (alpha or gamma; 18n3 or 6), docosapentaenoate (22:5n3), and palmitoyl sphingomyelin. Overall, patients with diabetes and periodontal disease demonstrated salivary metabolic signatures associated with increased purine degradation, decreased redox balance capacity, and altered omega-3/omega-6 fatty acid profiles.

The potential association of glioblastoma to periodontal disease was studied using metabolomic analyses.[99] Additionally, this study sought to identify differential metabolite profiles among periodontally healthy individuals, individuals with gingivitis/early periodontitis, and individuals with moderate/advanced periodontitis. Metabolites that were particularly important in diagnosing periodontal disease included caprocate, isocaprocate-butyrate, isovalerate, lactate + proline, and proline. Short-chain fatty acids such as butyrate, caprocate, isocaprovate, propionate, isovalerate, and lactate are end products of bacterial metabolism and showed a strong association to periodontal disease progression. However, no association between glioblastoma and periodontitis was found.

A site-specific analysis of metabolites in periodontal disease was carried out to determine the identities of differential metabolites at various stages of periodontal disease progression.[96] Gas chromatography-mass spectrometry was used to determine the metabolite composition in gingival crevicular fluid at both moderate and deep pocket depths. Moderate pocket depths demonstrated a metabolite profile that was intermediate between those of deep pocket depths and healthy sites. These metabolic shifts reflect a transitional profile that provides insight into potential early detection and diagnosis of periodontal disease at the molecular level. Putrescine, lysine, phenylalanine, ribose, taurine, 5-aminovaleric acid, and galactose were significantly increased in deep pocket sites in comparison with moderate pocket sites and healthy sites. There was a gradual moderate increase in lactic acid, benzoic

acid, glycine, malic acid, and phosphate from healthy to moderate pocket sites and from moderate to deep pocket sites. Unidentified metabolites were also noted to increase in a gradual manner consistent with periodontal disease severity, but these metabolites were not explicitly designated.

Mass spectrometry-based ionomics and targeted lipidomics on fatty acid metabolites were used to identify alterations in fatty acid metabolism and redox status in patients with chronic periodontitis.[100] Additionally, the patient's nutritional intake was analyzed to determine nutritional factors (specifically in terms of antioxidant vitamin intake) on periodontal disease status. An inverse association between antioxidant vitamin levels and periodontitis was observed, with decreased levels of vitamins A, C, and E among the periodontitis group. Ionomic profiling of plasma and saliva revealed significant decreases in redox-active metals in chronic periodontitis; an inverse relationship also emerged among trace metals with redox-modulating potential, including calcium, magnesium, zinc, copper, iron, and selenium. The effect of redox-active trace metals on local inflammation was analyzed using inductively coupled plasma-mass spectrometry-based ionomics to determine relative ion concentrations in saliva. Ions with a known association with antioxidant properties, including copper, manganese, and zinc, showed significantly reduced concentrations in the periodontitis group, suggesting increased oxidative stress in the local environment of periodontal inflammation. Additional ions that showed decreased concentrations in the periodontitis metabolic profile included potassium, magnesium, and calcium. Gas chromatography-mass spectrometry was used to quantify fatty acids in periodontitis. A significant decrease in C12:0 and C14:0 was observed in the periodontitis group. Lipidomics also revealed increased levels of cyclooxygenase products, including prostaglandin E_2, prostaglandin D_2, prostaglandin F2alpha, and thromboxane B2, as well as decreased levels of prostaglandin I_2, as metabolic markers of chronic periodontitis. Increased levels of 5-hydroxyeicosatetraenoic acid and decreased levels of 13-hydroxyeicosatetraenoic acid and 9-hydroxyeicosatetraenoic acid revealed differential lipoxygenase products of arachidonic acid and linoleic acid in chronic periodontitis. Additionally, significantly elevated levels of salivary F_2-isoprostanes, free radical lipid peroxidation products, and markers of oxidative stress were elevated in chronic periodontitis.

4.3 | Metabolomic microbial contributions

Recent efforts have been made to understand the contribution of bacterial metabolism to the salivary metabolic profile in periodontal disease. Salivary metabolomics was utilized to characterize the periodontal disease status in the presence and the absence of supragingival plaque using a pre- and postdebridement approach and gas chromatography-mass spectrometry.[101] Metabolites associated with periodontal inflammation included ornithine, 5-oxoproline, valine, proline, spermidine, hydrocinnamate, histidine, and cadaverine. Following debridement, a significant decrease in cadaverine was observed, as well as decreasing trends for ornithine, spermidine, and 5-oxoproline.

In summary, transcriptomic, proteomic, and metabolomic studies have provided a deeper understanding of periodontal disease pathogenesis at the molecular level. These studies have shed new light on the host-microbe interactions, processes, and pathways that underlie

periodontal disease, including modulation of the host immune response, tissue homeostasis, and complex metabolic processes of the host and oral microbiome. Further integration of these -omic approaches will enhance our ability to diagnose and treat various stages and forms of periodontal disease.

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TABLE 1

Human and animal transcriptomic studies

Reference	Transcriptomic focus	Periodontal condition	Findings
Lundmark et al (2018)[20]	Host	Periodontitis	Increased *IGLL5, SSR4, MZB1, XBP1*
Cho et al (2017)[9]	Host	Periodontitis with tobacco use	Methylation of extracellular matrix organization genes (*EGFL7, GPRC5C, IGF2, KLF9, PODN, RAD54L, STARD9*)
Horie et al (2016)[11]	Host	Periodontitis	Decreased extracellular matrix components (*DLX5, RUNX2*)
			Decreased transcription factors (*BARX1, PAX9, LHX8, DLX5*)
Taiete et al (2017)[10]	Host	Healthy tissue with periodontitis (chronic or aggressive) history	Increased natural killer cell receptors (*KIR2DL, KLRC1*) and immunity genes (*LILRA5, FCGR1A*)
			Decreased keratinocytes (*CASP14*)
Ebersole et al (2016)[13]	Host	Aging and periodontitis	Increased adaptive immune responses (*V-DX, V-DK, IGK, IGV, IGL*)
Kim et al (2016)[12]	Host	Chronic periodontitis	Increased defense/immunity genes (*CSF3, MAFA, CR2, GLDC, SAA1, LBP, MME, MMP3, MME-AS1, SAA4*)
			Decreased cytoskeletal genes (*SERPINA12, MT4, H19, KRT2, DSC1, PSORS1C2, KRT27, LCE3C, AQ5, LCE6A*)
Pandruvada et al (2016)[14]	Host	Aging and periodontitis	Increased osteoclastic function (*SPP1, TLR4, MMP8, TFEC*)
			Decreased osteoblastic activity (*SMEK3P, SMAD5*)
Jorth et al (2014)[16]	Bacteria	Periodontitis	Although disease-associated periodontal microbiota are similar to health-associated communities, disease-associated communities change metabolic gene expression
Lundmark et al (2015)[8]	Host	Periodontitis	Increased glycoprotein-producing gene *MUC4*
Santiago-Rodriguez et al (2015)[15]	Virus	Periodontitis	Increased viral gene expression (antirepressor, holin)
Becker et al (2014)[19]	Host	Peri-implantitis	27 transcripts separating peri-implantitis and periodontitis in cluster analysis
Duran-Pinedo et al (2014)[17]	Bacteria	Periodontitis	Increased *P. gingivalis* hemolysin genes, *T. denticola* flagella synthesis
			Increased metalloprotease, peptidase
Lakschevitz et al (2013)[18]	Host	Periodontitis	Increased neutrophil product gene expression (*BCL2*)
			Decreased *BAX* inducing apoptosis

AQP5 Aquaporin 5 AQ5; *BARX1*, BARX homeobox 1; *BAX*, BCL2 associated X, apoptosis regulator; *BCL2*, BCL2 apoptosis regulator; *CASP14*, caspase 14; *CR2*, complement C3d receptor 2; *CSF3*, colony stimulating factor 3; *DLX5*, distal-less homeobox 5; *DSC1*, desmocollin 1; *EGFL7*, EGF like domain multiple 7; *FCGR1A*, Fc fragment of IgG receptor 1a; *GLDC*, glycine decarboxylase; *GPRC5C*, G protein-coupled receptor class C group 5 member C; *IGF2*, insulin like growth factor 2; *IGK*, immunoglobulin kappa locus; *IGL*, immunoglobulin lambda locus; *IGLL5*, immunoglobulin lambda-like polypeptide 5; IGVH Immunoglobulin Heavy Chain region Genes *IGV*; *H19*, H19 imprinted maternally expressed transcript; *KIR2DL*, killer cell immunoglobulin like receptor, two Ig domains and long cytoplasmic tail 1; *KLF9*, kruppel like factor 9; *KLRC1*, killer cell lectin like receptor 1; *KRT2*, keratin 2; *KRT27*, keratin 27; *LBP*, lipopolysaccharide binding protein; *LCE3C*, late cornified envelope 3C; *LCE6A*, late cornified envelope 6A; *LHX8*, LIM homeobox 8; *LILRA5*, leukocyte immunoglobulin like receptor A5; *MAFA*, MAF BZIP transcription factor A; *MME*, membrane metalloendopeptidase; *MME-AS1*, MME antisense RNA 1; *MMP3*, matrix metallopeptidase 3; *MMP8*, matrix metallopeptidase 8; *MT4*, metallothionein 4; *MUC4*, mucin 4, cell surface associated; *MZB1*, marginal zone B and B1 cell specific protein; *PAX9*, paired box 9; *PODN*, podocan; *PSORS1C2*, psoriasis susceptibility 1 candidate 2; *RAD54L*, RAD54 like; *RUNX2*, RUNX family transcription factor 2; *SAA1*, serum amyloid A1; *SAA4*, serum amyloid A4, constitutive; *SERPINA12*, serpin family A member 12; *SMAD5*, SMAD family member 5; *SMEK3P*, protein phosphatase 4 regulatory subunit 3C;

SPP1, secreted phosphoprotein 1; *SSR4*, signal sequence receptor subunit 4; *STARD9*, StAR related lipid transfer domain containing 9; *TFEC*, transcription factor EC; *TLR4*, toll like receptor 4; *V-DK4-JH4b*; *V-DXP4-JH6c*; *XBP1*, X-box binding protein 1.

TABLE 2

Human, animal and in vitro proteomic studies

Reference	Study type	Periodontal condition	Sample type	Microbial species	Findings
Bostanci et al (2018)[32]	Human	Healthy, gingivitis, chronic periodontitis, aggressive periodontitis	Saliva		Increased MMP9, ras-related protein, actin Decreased clusterin, malignant brain tumor proteins
Marinho et al (2018)[31]	Human	Chronic periodontitis	Gingival crevicular fluid		Increased RGNEF, S100A8, S100A9, immunoglobulins Decreased actins, MARCKS, glutathione transferase
Batschkus et al (2018)[33]	Human	Chronic periodontitis	Gingival crevicular fluid		Trichloroacetic acid/acetone precipitation increased protein identification by 32%
Belstrom et al (2016)[34]	Human	Periodontitis	Saliva		Increased complement and coagulation cascades
Monari et al (2015)[35]	Human	Periodontitis	Periodontal pocket tissue		Increased S100A9, HSPB1, LEG 7, and 14-3-3
Trindade et al (2015)[36]	Human	Chronic periodontitis	Saliva		Increased metalloproteases, MMP9
Bertoldi et al (2013)[40]	Human	Periodontitis	Periodontal pocket tissue		Increased annexin A2, actin cytoplasmic 1, carbonic anhydrase 1&2, immunoglobulin kappa chain C region and flavinreductase Decreased 14-3-3, heat-shock protein, triosephosphateisomerase, peroxiredoxin, fatty acid binding protein, galetin 7
Salazar et al (2013)[39]	Human	Periodontitis	Saliva		20 proteins increased by 1.5-fold
Silva-Boghossian et al (2013)[37]	Human	Chronic periodontitis	Gingival crevicular fluid		43 proteins only detected in periodontitis: 10 for cell differentiation, 3 for cell organization, 1 for coagulation, 8 for immune response, 9 for metabolism, 8 for signal transduction, 2 for transport, 2 no specific function
Baliban et al (2012)[41]	Human	Chronic periodontitis	Gingival crevicular fluid		Human proteins: neutrophil defensin, carbonic anhydrase, elongation factor Bacterial proteins: 33 kDa, iron uptake, phosphorenolpyruvate carboxylase, ribulose biphosphate carboxylase, coenzyme A transferase
Carneiro et al (2012)[29]	Human	Healthy	Gingival crevicular fluid		In healthy proteome data, 57% of proteins can be defined in plasma, 43% in gingival crevicular fluid uniquely
Range etal (2012)[38]	Human	Periodontitis in obesity	Saliva		Increased albumin, hemoglobins, defensin (defensin uniquely in obesity and periodontitis)
Choi et al (2011)[43]	Human	Moderate periodontitis	Gingival crevicular fluid		Increased azurocidin
Goncalves et al (2010)[47]	Human	Gingivitis	Saliva		Increased serum albumin, hemoglobulin, immunoglobulin, keratin
Heo et al (2011)[45]	Human	Healthy, gingivitis, moderate	Gingival crevicular fluid		Increased S100A2, S100A8, S100A9

Reference	Study type	Periodontal condition	Sample type	Microbial species	Findings
		periodontitis, severe periodontitis			
Mizuno et al (2011)[46]	Human	Aggressive periodontitis, chronic periodontitis	Blood (neutrophils)		Increased lactoferrin, caldesmon, heat-shock protein and stac
Bostanci et al (2010)[48]	Human	Generalized aggressive periodontitis	Gingival crevicular fluid		Increased PMNs, MMP8, cathepsin, myeloperoxidase
Goncalves et al (2010)[47]	Human	Gingivitis	Saliva		Increased albumin, hemoglobin and immunoglobulin Decreased cystatin
Haigh et al (2010)[49]	Human	Severe periodontitis	Saliva		Increased S100A8, S100A9, S100A6, haptoglobin, prolactin, parotid secretory protein
Bostanci et al (2018)[30]	Primates	Chronic periodontitis	Gingival crevicular fluid		Cp40 downregulated periodontitis-related proteins: C09, C05, C03, CFAH, C08B, IC1, C04A, IGHM, IGHA2, IGHG1, C07
Davis et al (2016)[65]	Canine	Progression from gingivitis to mild periodontitis	Gingival crevicular fluid		Increased haptoglobin
Huynh etal(2015)[61]	Canine	Healthy, gingivitis, and chronic periodontitis	Gingival crevicular fluid		Increased complement, immunoglobulins, keratin, fibronectin, lactotransferrin, 14-3-3, defensin, actin
					Decreased cystatin
Veith et al (2018)[66]	In vitro			*P. gingivalis*	In heme limitation: binding and transporting heme proteins were increased
					In heme excess: heme efflux proteins were increased
Bao et al (2017)[68]	In vitro			*A. actinomycetemcomitans*	Increased ribosomal origin, proteolysis, carbon metabolism, iron transport
Bengtsson et al (2017)[69]	In vitro			*P. gingivalis*	PLNC8 increased growth factors, cell proliferation, decreased apoptosis
Llama-Palacios et al (2017)[67]	In vitro			*A. actinomycetemcomitans*	Increased YeaT, FtsZ, OMP39, OMP18/16, chaperone, OMPA, adenyldate kinase, dihydrolipoamide acetyltransferase
Kieselbach et al (2015)[73]	In vitro			*A. actinomycetemcomitans*	Outer membrane proteins activate innate immunity through NOD active pathogen pathways
Stobernacket al (2016)[70]	In vitro			*P. gingivalis*	Increased citrullinating enzymes to target bacterial and human proteins
Preiano et al (2016)[71]	In vitro			Healthy biofilm	Decreased HNP-3, S100A8, S100A9 in health, suggesting S100A as a diagnostic biomarker
Bao et al (2015)[72]	In vitro			Complex	Increased cytoskeletal, stress, apoptosis, antigen presentation, biofilm lysate proteins
Bostanci et al (2015)[74]	In vitro			Complex	Increased inflammation and apoptosis Decreased tissue turnover
Kieselbach et al (2015)[73]	In vitro			*A. actinomycetemcomitans*	Increased LtxA, putative virulence-related proteins
Ogita etal (2015)[75]	In vitro			Healthy biofilm	Increased galectin-7 for wound healing after laser treatment

Reference	Study type	Periodontal condition	Sample type	Microbial species	Findings
Veith et al (2018)[66]	In vitro			*P. gingivalis*	Increased CTD proteins and virulence factors at the outer membrane
Cogo et al (2012)[80]	In vitro			*P. gingivalis*	After exposure to nicotine, increased phosphormannomutase, spot, OxyR, rubrerythrin, RagA, prostaglandin 50, aminopeotidase, peptidase, elongation factor, RRF, Rho
Kishi et al (2012)[77]	In vitro			*P. gingivalis*	Increased prostaglandin N0743, prostaglandin N0876, prostaglandin N1513, and prostaglandin N0729
Zainal-Abidin et al (2012)[79]	In vitro			Complex- *P. gingivalis, T. denticola* and *T. forsythia*	Increased HusA, HusB Decreased HmuY
Zijnge etal (2012)[78]	In vitro			*A. actinomycetemcomitans*	Increased extracellular virulence proteins
Pham et al (2010)[81]	In vitro			*T. forsythia*	Increased lipoproteins, TonB, oxidative stress response Decreased butyrate, pyruvate, metabolism, amino acid biosynthesis, trichloroacetic acid cycle

14-3-3, 14-3-3 protein; CFAH, complement factor H; CO3, cytochrome *c* oxidase subunit 3; CO5 complement component C5; CO7 complement component C7, complement component C7 precursor; CO9 complement component C9a; CO4A complement component C4-A; CO8B complement component C8 beta chain; CTD, C-terminal domain; FtsZ cell division protein stands for “Filamenting temperature-sensitive mutant Z”; IC1 plasma protease C1 inhibitor; HmuY a novel heme-binding protein of Porphyromonas gingivalis, stands for “hemin utilization protein”; HNP-3, neutrophil defensing 3; HSPB1, heat shock protein family B; HusA Hemin uptake system protein A; HusB Hemin uptake system protein B; IGHA2, immunoglobulin heavy constant alpha 2; IGHG1, immunoglobulin heavy constant gamma 1; IGHM, immunoglobulin heavy constant mu; LEG 7 Galectin 7; LtxA, leukotoxin; MARCKS, myristoylated alanine-rich C-kinase substrate; MMP-8, neutrophil collagenase; MMP-9, matrix metalloproteinase-9; NOD nucleotide-binding oligomerization domain; OMP18/16, outer membrane protein 18/16; OMP39, outer membrane protein 39; OMPA outer membrane protein A; OxyR redox-sensitive transcriptional activator, PLNC8, plantaricin NC8; PMN, polymorphonuclear leukocyte; RagA transport and binding activity RagA protein, Ras-related GTP-binding protein A; RGNEF, Rho guanine nucleotide exchange factor 28; RRF, ribosome releasing factor; S100A protein S100A; S100A2, protein S100-A2; S100A6, protein S100-A6; S100A8, protein S100-A8; S100A9, protein S100-A9; TonB, protein TonB; YeaT.

TABLE 3

Human metabolomic studies

Reference	Sample type	Periodontal condition	Analysis method	Findings
Chen et al (2018)[91]	Gingival crevicular fluid	Generalized aggressive periodontitis	GC-MS	Oxidative stress: increased dehydroascorbic acid, urea, xanthine; decreased glutathione
				Purine degradation: increased xanthine and urea
				Tyrosine metabolism: increased noradrenaline
				Pyrimidine metabolism: increased beta-alanine, uridine, malonate; decreased thymidine
				Bacterial biochemistry: increased ribulose-5-phosphate, glucose-1-phosphate
Garcia-Villaescusa et al (2018)[99]	Saliva	Moderate and severe periodontitis	H-NMR	Early periodontitis: increased lactate-proline, lactate, proline; decreased caprocate, isoleucine, isopropanol, choline
				Moderate-severe periodontitis: increased caprocate, isoleucine, isopropanol, choline, isocaprocate + butyrate, isovalerate, 4 aminobutyrate; decreased sucrose, sucrose-glucose-lysine, lactate-proline, lactate, proline
Romano et al (2018)[94]	Saliva	Chronic periodontitis	H-NMR	Chronic periodontitis: decreased pyruvate, *N*-acetyl groups, lactate; increased tyrosine, valine, isoleucine, phenylalanine, proline
		Aggressive periodontitis		Aggressive periodontitis: decreased pyruvate, *N*-acetyl groups, lactate, sarcosine; increased tyrosine, phenylalanine, formate
Rzeznik et al (2017)[98]	Saliva	Active periodontitis (chronic and aggressive)	H-NMR	Increased butyrate (short-chain fatty acids)
				Decreased fucose, lactate, acetate, *N*-acetyl, gamma aminobutyrate, 3-D-hydroxybutyrate, pyruvate, methanol, threonine, ethanol
Sakanaka et al (2017)[97]	Dental plaque	Low and high PISA (pre- and postdebridement)	GC-MS	High PISA (predebridement): 4 aminobutyric acid, 5-oxoproline, cadaverine, hypotarine, phenylalanine, aspartic acid, 5-amino-valeric acid, succinic acid, indole-3-acetic acid, glutamic acid, alanine-3TMS, putrescine, leucine, *N*-acetylornithine, hydrocinnamate, ornithine, fucose-2, fructose-6-phosphate.
	Saliva			High PISA (decreased by debridement): 4-aminobutyric acid, cadaverine, phenylalanine, 5-aminovaleric acid, succinic acid, putrescine, hydrocinnamate, ornithine, fructose-6 phosphate
				Low PISA (predebridement): 5-oxoproline, aspartic acid, tryptophan, glutamine, fucose-2, glutamic acid, indole-3-acetic acid, *N*-acetylornithine, isoleucine-1TMS, fucose-1, ethanolamine, leucine, alanine-3TMS, hypotaurine, alanine-2TMS
				Low PISA (decreased by debridement): tryptophan, glutamine, isoleucine-1TMS, fucose-1, ethanolamine, alanine-2TMS
Kuboniwa et al (2016)[101]	Saliva	Periodontitis	GC-MS	Supra-/subgingival: increased ornithine, 5-oxyproline, valine, spermidine, hydrocinnamate, histidine, cadaverine
				Sub- vs supragingival (postdebridement): decreased cadverine ornithine, spermidine, 5-oxoproline
Ozeki et al (2016)[96]	Gingival crevicular fluid	Moderate pocket depth	GC-MS	Moderate pocket depth: increased propylamide, lactic acid, benzoic acid, glycine, phosphate, succinic acid, alanine, malic acid, glutamic acid, 5-aminocaleric acid, phenylalanine, inositol, octadecanoate; decreased hydrocinnamate

Reference	Sample type	Periodontal condition	Analysis method	Findings
		Deep pocket depth		Deep pocket depth: increased propylamide, lactic acid, benzoic acid, phosphate, glycine, succinic acid, alanine, hydrocinnamate, malic acid, glutamic acid, 5-aminovaleric acid, phenylalanine, ribose, taurine, putrescine, galactose, lysine, inositol, octadecanoate
Barnes et al (2011)[95]	Saliva	Healthy, gingivitis, and periodontitis	LC-MS and GC-MS	Diabetic saliva and plasma: decreased 1–5 anhydroglucitol, increased glucose, increased alpha-hydroxybutyrate
	Plasma			Saliva periodontitis vs healthy: increased purine degradation (oxidative stress), dipeptides (macromolecular degradation of proteins), amino acid metabolites (p-cresol sulfate), carbohydrates (monosaccharides indicative of amylase activity), energy metabolites (TCA, indicative of energenic stress), uridine (DNA/RNA degradation), allanation, omega-6 fatty acids (link to inflammation), fatty acids, acetylcarnitine, carnitine, 3-dehydrocarnitine
				Diabetic gingivitis vs diabetic healthy (saliva): (purine degradation) increased adenosine, inosine, guanine, guanosine, xanthine, glutathione (oxidized), cysteine-glutathione disulfide
				Diabetes and periodontal disease (fatty acids and sphingomyelins): 12-HETE, linoleate (18:2n6), linoleate (alpha or gamma 18n3 or 6), docosapentaenoate (n3 DPA, 22:5n3), arachidonate (20:4n6), palmitoyl sphingoyelin
Huang et al (2014)[100]	Saliva	Chronic periodontitis	GC-MS	Redox status: decreased vitamin A, vitamin B1, vitamin B2, vitamin PP, vitamin C, vitamin E, Ca, Mg, Cu, Fe, Se, Zn, K, Ca, Mn
				Fatty acids: decreased C12:0, C14:0
				Aracidonic acid metabolites: increased prostaglandin D2, prostaglandin E2, prostaglandin F2alpha, thromboxane B2, 5-HETE; decreased prostaglandin I2, 9-HODE, 13-HODE

5-HETE, 5-hydroxyeicosatetraenoic acid; 12-HETE, 12-hydroxyeicosatetraenoic acid; 9-HODE, 9-hydroxyoctadecadienoic acid; 13-HODE, 13-hydroxyoctadecadienoic acid; Ca, calcium; Cu, copper; DPA, docosapentaenoic acid; Fe, iron; GC-MS, gas chromatography-mass spectrometry; H-NMR, proton nuclear magnetic resonance; K, potassium; LC-MS, liquid chromatography-mass spectrometry; Mg, magnesium; Mn, manganese; PISA periodontal inflamed surface area; TCA, tricarboxylic acid; Se, selenium; Zn, zinc.