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1 | INTRODUCTION

Environment refers to the surroundings or conditions as a whole in which an organism lives. Environment matters for any living organism independent to the size or nature of the organism; it can be a microorganism such as bacteria or a macroorganism such as a human. As part of the environment, numerous factors affect the physiology and functions of the living body. This is also true for the complex interactions which occur between macro- and microorganisms.

Periodontal diseases are among the most common chronic infectious diseases worldwide. Periodontitis remains one of the major causes of tooth loss in the adult population. Subgingival bacteria and their toxins are the primary etiological factor of periodontitis, however, the tissue destruction which occurs is a consequence of host response activities that are triggered by microbial factors. Periodontitis is regarded as a "dysbiosis". Dysbiosis is defined as a condition in which the balanced state of the ecosystem is disturbed.[1] These disturbances usually correspond to external pressures such as states of disease or medications. Disruption of the finely tuned equilibrium of the bacterial ecosystems in the human microbiome is associated with a plethora of diseases. This is also true for the pathogenesis of periodontitis, as there are always bacteria in the oral cavity, but in health there is a balance between "good" and "bad" bacteria in the dental biofilm. In healthy periodontium, the oral microbiota and the host exist in a symbiotic state; the colonization of pathogenic microorganisms is prevented and resident bacteria contribute to the host physiology. Moreover, microbial attacks are successfully handled by the host response, thus maintaining a healthy state. However, when there is dysbiosis, the attacks may be too strong, the defense may be insufficient, or both of these can be present concurrently. Resilience, on the other hand, is defined as the ability of the host to recover from a state of disease.[2] The ability of an ecosystem to rebound from the dysbiotic state and reestablish a health-compatible community plays an important role in its susceptibility to disease in the future. There is a wide range of variability among different individuals in terms of how tolerant one can be to disturbances in the environment, and some are more capable of returning more quickly to their original balanced state than a less resilient individual.[3] The mechanistic underpinnings of a shift to a dysbiotic community remain poorly understood.

In 1683, using a microscope he had invented, Antony van Leeuwenhoek observed bacteria for the first time, and that first specimen was dental plaque.[4] Since then, tremendous progress has occurred in those techniques used for microbiologic analysis. Currently, omics studies focusing on the microbiome are key to unraveling both the location and role of microorganisms in human diseases. Microbiome refers to the totality of microbes, their genetic information, and the environment in which they interact.[5] Microbiota is the term for all of those microbial organisms that integrate the microbiome.[1] The oral microbiome comprises bacteria, viruses, fungi, protozoa, and archaea.[6] More than 700 different microorganisms have been identified in the oral cavity to date. Not surprisingly, the oral microbiome is considered to one of the most complex bacterial flora in the human body.[7] Because of the high potential for various environmental factors to affect the composition of the oral microbiome, it is regarded as remarkably dynamic. The relative proportions of various bacterial species are affected by interbacterial interactions and by factors associated with modern life such as sugar consumption, the use of antibiotics and other antimicrobials, and vaccines.[8] Diet, dietary supplements, tobacco products, and psychological stress are the major environmental factors affecting the composition of the oral microbiome as well as the interactions among various microorganisms (Figure 1). A reciprocal and dynamic balance between the human host and its microorganisms determines oral health.[9]

2 | TOBACCO PRODUCTS AND PERIODONTAL MICROBIOME

Smoking is the strongest "modifiable/preventable" risk factor for periodontitis. Despite a decrease in smoking habits, it is estimated that ~10% of all deaths in 2020 will be related to smoking.[10] On the other hand, severe periodontitis affects ~700 million people worldwide. Without any smokers, the risk of periodontitis would be reduced by 14%.[11]

For the first time, 50 years ago, Pindborg[12] reported that smoking had a detrimental effect on oral health. Since then, clinical studies have reported clinical, biochemical, and microbiologic findings linking tobacco products with the extent and severity of periodontal diseases. The World Health Organization defines a "smoker" as someone who smokes any tobacco product, either daily or occasionally. The extent and severity of periodontal disease is much higher in smokers compared with nonsmokers and smokers are more susceptible to tooth loss.[13] The clinical signs and symptoms of gingival inflammation are suppressed in smokers as a consequence of vascular changes.[14] As a result, gingival hyperemia and bleeding are less visible in these patients, possibly reducing their demand for periodontal treatment.[15] Moreover, smokers have a higher risk of unresponsive pockets and further breakdown during supportive periodontal treatment,[16] which will eventually increase their treatment costs.[17] In general, smokers are less compliant than nonsmokers.[18,19] Patients who smoke present less reduction in probing depth and less clinical attachment gain with nonsurgical periodontal treatment.[20,21] Surgical periodontal treatment provides less reduction in probing depth and less clinical attachment gain.[22] Although findings from different studies are controversial, smoking seems to negatively affect the outcomes of root closure surgeries. Failure rates of dental implants are higher in smokers and smokers have a higher risk of postoperative infections and marginal bone loss.[23]

In smokers, pathogen bacteria can more frequently colonize shallow sites. Smoking may lead to a shift in subgingival biofilm, increasing the prevalence of pathogens as well as decreasing the rate of commensal microbial populations. It is also evident that patients with periodontitis who smoke show less of a decrease in the rates of pathogens following nonsurgical periodontal treatment. It is likely that decreased local oxygen tension caused by smoking may promote the growth of anaerobic bacteria. Moreover, bacteria can easily adhere to epithelial cells in smokers and this is a crucial step for bacterial aggregation.

The possible effects of smoking on the periodontal microbiome have been investigated in various studies using different laboratory techniques. Because the subgingival microbiome is largely uncultivated, cultivation-based and targeted molecular approaches have limited value in evaluating the relationship between smoking and oral microbiome. Clinical research conducted in humans, by either cross-sectional or intervention studies, experimental animal models, and in vitro studies, all provide complementary data on this issue.

2.1 | Clinical studies

Smokers are regarded as more susceptible to periodontal diseases and more likely to be infected with *Porphyromonas gingivalis* than nonsmokers. Moreover, smoking causes alterations in the expression of surface components resulting from this bacterium. The overall IgG response to *P. gingivalis* and the in vivo reflections of tobacco-induced phenotypic changes in this bacterium have been investigated.[24] The humoral immune response to *P. gingivalis* strains and specific tobacco-regulated outer membrane proteins (FimA and RagB) was evaluated by ELISA in smoking and nonsmoking patients with chronic or aggressive periodontitis. The presence of local or systemic DNA of *P. gingivalis* was also evaluated by PCR. Smokers exhibited a decreased total IgG response to the clinical strains of *P.*

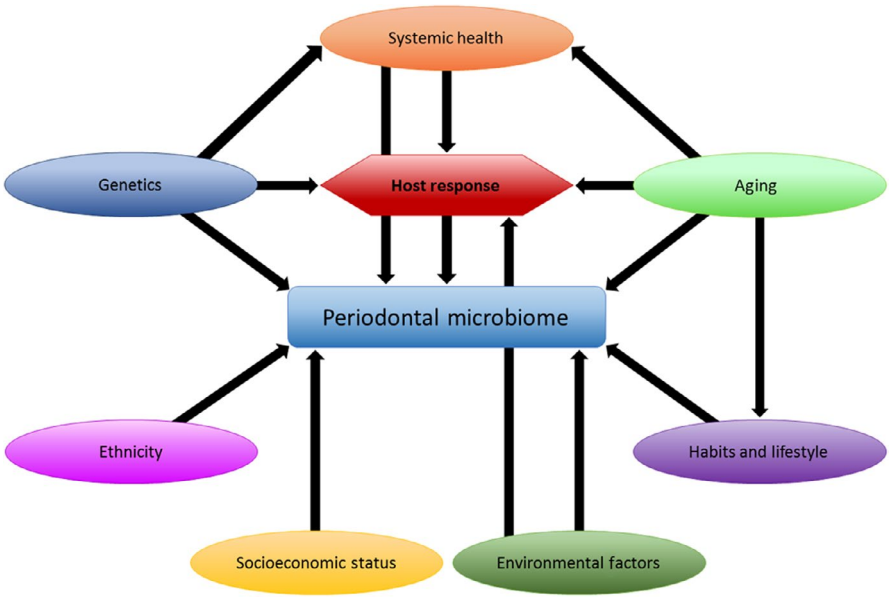

FIGURE 1 Complex interactions between host response, environmental factors, and microbiota acting in the etiopathogenesis of periodontal diseases [Colour figure can be viewed at wileyonlinelibrary.com]

gingivalis. As determined by 16S ribosomal RNA analysis, seropositive smokers were more likely to be infected orally and systemically with *P. gingivalis*. These findings indicate alterations in the smoking-related humoral response against *P. gingivalis*, possibly reflecting differences in the pathogenic mechanisms of periodontal disease progression between smokers and nonsmokers.

To compare the presence of periodontopathogen bacteria in patients with chronic periodontitis, they were divided into groups of nonsmokers and light, average, and heavy smokers.[25] The detection rates of *Tannerella forsythia*, *P. gingivalis*, and *Prevotella intermedia* were higher in the groups of smokers than in the groups of nonsmokers, indicating a clear relationship between smoking and the presence of the pathogenic microbial profile. In a large study of American adults, a possible relationship between cigarette smoking and the oral microbiome was investigated.[26] Oral wash samples were analyzed by 16S ribosomal RNA gene sequencing and the overall microbiome composition significantly differed between current smokers and those who had never smoked. The genera *Capnocytophaga*, *Peptostreptococcus*, and *Leptotrichia* were depleted, whereas *Atopobium* and *Streptococcus* were enriched in those who were current smokers compared with those who had never smoked. These effects were related to changes in carbohydrates and energy metabolism together with those to xenobiotic metabolism, suggesting that smoking-related alterations in the oral microbiome may possibly lead to shifts in functional pathways.

Other studies using a checkerboard DNA-DNA hybridization technique reported a similar bacterial profile in the subgingival plaque samples in cigarette smokers, waterpipe smokers, and nonsmokers.[27,28]

The composition of subgingival plaque samples obtained from patients with moderate chronic periodontitis who were smokers and nonsmokers was investigated in a Korean population using 16S ribosomal RNA sequencing.[29] The genera *Fusobacterium*, *Fretibacterium*, *Streptococcus*, *Veilleonella*, *Corynebacterium*, and *Filifactor* were abundant in smokers. The less abundant species in smokers were *Prevotella*, *Campylobacter*, *Aggregatibacter*, *Veillonellaceae*, *Haemophilus*, and *Prevotellaceae*. Species richness and evenness were similar in both the smoking and nonsmoking groups, with smokers exhibiting a greater diversity than nonsmokers. The observed differences in the bacterial community suggest a significant influence of cigarette smoking on subgingival bacterial ecology. The presence and number of periodontal pathogens in the subgingival microbiota of patients with chronic periodontitis who smoke were compared with those of their nonsmoking counterparts using real-time PCR.[30] Full-mouth clinical periodontal examinations and subgingival plaque sampling were performed in 40 current smokers and in 40 individuals who had never smoked. Smoking status was confirmed by measuring expired carbon monoxide concentrations with a carbon monoxide monitor. Real-time PCR was used to detect and quantify *Aggregatibacter actinomycetemcomitans*, *P. gingivalis*, *T. forsythia*, and *Treponema denticola*. Patients who smoked revealed higher values of probing depth and clinical attachment level measurements, and fewer sites with bleeding on probing. Smoking status was found to be associated with the presence of *A. actinomycetemcomitans*. Moreover, counts of *A. actinomycetemcomitans*, *P. gingivalis*, and *T. forsythia* were significantly higher in patients who smoked than in nonsmokers. On the other hand, with culture and quantitative PCR techniques, no difference was found between patients with periodontitis who smoked and those did not smoke with regard to the composition of subgingival microbiome.[31] However, the 16S sequencing used in the same study revealed significant intergroup differences, as the operational taxonomic units classified to *Fusobacterium*, *Prevotella*, and *Selenomonas* were more abundant in smokers, whereas those belonging to the genera *Peptococcus* and *Capnocytophaga* were more abundant in nonsmokers. The authors related low taxonomic diversity with higher disease severity, particularly in smokers. These findings provide further support for the hypothesis that ecological factors play an important role in host-microbiome interactions. Moreover, this study emphasizes the importance of the assay technique for the sensitivity of microbiologic analysis as conventional techniques may fail to detect the intergroup differences which can be found by novel technologies.

The effects of smoking on colonization dynamics and resilience in marginal and subgingival biofilms were evaluated in patients with preexisting gingivitis who were smokers and nonsmokers.[2] Marginal and subgingival plaque samples were obtained from 25 current smokers and 25 patients who had never smoked and the samples were analyzed using 16S cloning and sequencing. Patients who smoked demonstrated an early pathogenic colonization that resulted in sustained pathogen enrichment with periodontal pathogens. Moreover, the abundance of pathogenic species was greater, and the compositional correlation between marginal and subgingival ecosystems was poorer in smokers. These findings indicate that smoking decreases the ability of subgingival microbiome to “reset” itself following episodes of disease, thereby lowering the resilience of the ecosystem and decreasing its resistance to future disease. In a previous experimental gingivitis study conducted by the same group, the onset of clinical signs of gingivitis in smokers was preceded by an increase in the levels of periodontopathogens such as *Preponema*, *Selenomonas*, *Parvimonas*, *Dialister*, and *Campylobacter*.[32] In smokers, both marginal and subgingival biofilms were characterized by early acquisition of pathogenic bacteria.

Another study compared subgingival bacterial communities using next-generation sequencing in 22 nonsmoking periodontally healthy individuals, 28 nonsmoking periodontal patients, and 32 periodontal patients who were smokers.[33] The microbial communities of patients with periodontitis who smoked were distinct from those of nonsmokers, supporting the hypothesis that smoking can directly affect the host's ability to control infection and, consequently, elimination of or control of microbiota, particularly “unusual” species, becomes more difficult, eventually increasing the risk of treatment failing.[34] In patients with periodontal disease who were smokers, Bizzarro et al[35] detected a higher proportion of *Fusobacterium*, and novel genera such as *Paludibacter* and *Desulfobulbus* were particularly evident. In patients with periodontitis who were smokers, Camelo-Castillo

et al[33] more frequently detected species of *Anaeroglobus* and *Bulleidia extructa*.

Bacterial profiles of saliva were investigated in a Danish adult population with a low level of periodontitis, and *Streptococcus sobrinus* and *Eubacterium* were found to be more associated with smokers than nonsmokers.[36] Differences in socioeconomic levels were also reflected in the salivary bacterial profiles. Socioeconomic status is a significant factor that has indirect effects on environmental conditions as well as on an individual's perceptions regarding the importance of oral health, using home care products, and seeking early diagnosis and treatment for oral health problems. Moreover, socioeconomic status has a direct influence on the level of access to health care.

In a recent study, shifts in salivary microbiota were evaluated comparatively in smokers and nonsmokers in Jordan.[37] High-throughput next-generation sequencing for V3-V4 hypervariable regions of the 16S ribosomal RNA gene was used and *Streptococcus*, *Prevotella*, *Veillonella*, *Rothia*, *Neisseria*, and *Haemophilus* were investigated. *Neisseria* and *Haemophilus* predominated the salivary microbiota of all samples, whereas there were increased levels of *Streptococcus*, *Prevotella*, and *Veillonella* in smokers. The authors suggested that there is a microbial signature at the genera level that can be used to classify smokers and nonsmokers by linear discrimination analysis effect size based on the salivary abundance of genera. However, the oral cavity is an open system which is continuously exposed to various intrinsic and extrinsic sources, and therefore it is a challenge to determine whether existing colonies in saliva are a long-term diversity or not.

Gram-negative and anerobic bacteria proliferate rapidly in patients with periodontitis. Subgingival microbiota from patients with chronic periodontitis who were smokers or nonsmokers were compared with samples from periodontally healthy individuals by 16S ribosomal RNA sequencing.[38] Patients who smoked revealed a major alteration in microbial communities, suggesting that there was an association between the severity of subgingival dysbiosis and smoking. Clearly, the dysbiosis or the tobacco-induced changes in the equilibrium of subgingival microbiota contribute to the severity of periodontal destruction. Subgingival plaque composition and clinical periodontal parameters were compared in patients with periodontitis who were smokers and nonsmokers.[39] The PCR technique was used for detection of *A. actinomycetemcomitans*, *P. gingivalis*, *T. forsythia*, *P. intermedia*, *Fusobacterium nucleatum/periodonticum*, *T. denticola*, and *Campylobacter rectus* in subgingival plaque samples and a relevant association between smoking and colonization by specific periodontal pathogens including *C. rectus* was reported. A statistically significant relationship was found between a probing depth of ≥4 mm and detection of *T. denticola*, *P. intermedia*, *T. forsythia*, or *C. rectus*. Moreover, bleeding on probing and detection of *C. rectus* or *P. intermedia* and smoking were found to be significantly associated.

Although there were differences among individuals, smokers with periodontitis revealed a robust core microbiome consisting of species identified in at least 80% of individuals, in which anaerobic bacteria dominated.[40] Smokers exhibited sparse co-occurrence of microbial networks that were predominantly congeneric. Another study investigating the possible effects of smoking on oral and nasal microbial diversity reported that cigarette smoking has the potential to change the microbial diversity and composition of the buccal mucosa.[41]

The composition of subgingival plaque samples and their relationship with clinical periodontal findings were compared among teenage smokers of both genders.[42] Periodontal bacteria were associated with higher periodontal index scores in both genders, and certain periodontal bacteria were found more frequently in females who smoked than in those who were nonsmokers. Differences between the genders such as hormone content and levels, circadian rhythm, and social habits may well have an influence upon the outcomes of environmental factors.

The microbial profile of periodontitis associated with smoking is distinct from that in nonsmokers, as there are significant differences in the prevalence and abundance of disease-associated and health-compatible bacteria.[43] Patients with periodontitis who smoke exhibit subgingival microbial communities that are less diverse than those of nonsmokers. Possible contributions of smoking to the composition and pro-inflammatory characteristics of biofilm were explored during de novo plaque formation in current smokers and individuals who had never smoked.[44] Smokers demonstrated a highly diverse, relatively unstable initial colonization of both marginal and subgingival biofilms compared with nonsmokers. These findings suggest that smoking favors the early acquisition and colonization of pathogens in oral biofilms. Tobacco smoke, which contains thousands of chemicals, promotes biofilm formation by increasing the adherence of bacteria to a stratum.[45] Nicotine, one of the major chemical components of tobacco smoke, can enhance dual-species biofilm formation with *Streptococcus mutans* and *Streptococcus sanguinis*.[46]

Passive smoking, also known as environmental smoking or second-hand smoking, can also harm periodontal tissues. Parents who smoke harbor more potential pathogens and fewer interfering organisms, and therefore they may act as a source of pathogens which can colonize and/or infect their children.[47]

Currently, 16S ribosomal RNA is the most efficient available approach with which to study microbial communities in terms of the possible effects of environmental factors such as smoking; however, it suffers from mosaicism, intra-genomic heterogeneity, and lacks a universal threshold of sequence identity value.[48]

2.2 | Response to periodontal treatment

The response to periodontal treatment has also been compared between smokers and nonsmokers with severe periodontal disease. Fifteen current smokers and 15 nonsmokers with chronic periodontitis received nonsurgical periodontal treatment comprising of conventional scaling and root planing, which was completed over six visits.[49] Subgingival plaque samples were obtained at baseline, then

immediately after scaling and root planing at 6 weeks, 9 weeks, and 6 months. The checkerboard DNA-DNA hybridization technique was used for analyzing the microbial plaque samples. The findings indicated that scaling and root planing resulted in a significant reduction in the mean counts of the three pathogens from the red complex, *Eubacterium nodatum*, but only of *Parvimonas micra* in the nonsmokers. The proportion of host-compatible species increased significantly in both smokers and nonsmokers from baseline to 6 months posttreatment. However, the proportion of pathogenic species only decreased significantly in the nonsmokers, suggesting that smokers exhibit recolonization of pathogenic subgingival biofilm more quickly than nonsmokers.

Smokers tend to respond less favorably to periodontal treatment than nonsmokers. Investigation of 40 bacterial species using checkerboard DNA-DNA hybridization indicated that the adjunctive effect of a single episode of antimicrobial photodynamic therapy to scaling and root planing failed to demonstrate microbiological improvements in smokers.[50] In another study, subgingival microbiologic changes with supragingival periodontal therapy were evaluated in smokers and nonsmokers presenting with severe chronic periodontitis.[51] The baseline data revealed that smokers harbored a higher proportion of *Porphyromonas endodontalis*, *Bacteroidetes* sp., *Fusobacterium* sp., and *T. forsythia*, together with lower numbers of cultivated phylotypes. Moreover, supragingival periodontal treatment only slightly affected the diversity of subgingival microbiota in smokers compared with nonsmokers.

The effects of active periodontal treatment and outcomes after 12 months of follow-up of supportive periodontal treatment were compared between 25 patients with periodontitis who smoked and 25 patients with periodontitis who were nonsmokers.[52] Smoking status was validated by measuring serum levels of cotinine, and when the checkerboard DNA-DNA hybridization technique was used to compare the subgingival microbial profile, smoking was found to be associated with the presence and abundance of red complex bacteria. Another recent study comparing clinical outcomes, biochemical findings, and subgingival microbiota between patients with periodontitis who were smokers and nonsmokers revealed significant differences in favor of Gram-negative bacteria for early colonization after completion of scaling and root planing.[53] This early colonization creates a much higher risk of recurrence of periodontitis in smokers. Smoking affects human microbiome directly, or indirectly via immunosuppressive mechanisms, oxygen deprivation, or biofilm formation. There is evidence of microbial dysbiosis in many diseases including periodontitis in a smoky environment, but the causal relationship between microbiome alterations and disease progress remains enigmatic.[48]

2.3 | Dental implants and smoking

The microbiota around dental implants show a great similarity to that of natural teeth. Clinical, microbiologic, and host-response parameters were compared between heavy smokers and nonsmokers with healthy implants.[54] The clinical parameters comprised the modified gingival index, the modified plaque index, and probing depth. Total bacterial load, as well as major periodontopathogenic bacteria (*T. forsythia*, *T. denticola*, and *P. gingivalis*) were investigated. The clinical parameters were worse in smokers than nonsmokers but without statistically significant differences. The microbiota in smokers consisted of greater numbers of these bacteria, however, the differences between both groups were not statistically significant. Thus, the authors concluded that smoking alone did not significantly affect the microbiologic parameters in healthy implants.

Peri-implant microbiome was compared between sites with healthy implants, peri-implantitis, and peri-implant mucositis using 16S ribosomal RNA sequencing, and lower diversity was reported in smokers.[55]

2.4 | Cessation of smoking and periodontal microbiome

Few studies to date have investigated the subgingival microbial profile in patients who have stopped smoking as a consequence of periodontal treatment. It has been suggested that cessation of smoking alters subgingival microbiota. In a longitudinal study, Delima et al[56] used molecular approaches for bacterial identification and enumeration to investigate the possible effects of smoking cessation on the prevalence and levels of selected subgingival bacteria. Subgingival plaque samples were obtained at baseline and then 1 year after nonsurgical periodontal treatment accompanied by smoking cessation counseling. Compared with those subjects who continued smoking, those who successfully stopped smoking for 1 year had subgingival microbiome that was colonized by a greater number of health-associated species and less putative periodontal pathogens. In another longitudinal study with a 1-year follow-up after nonsurgical periodontal treatment, smokers exhibited a similar bacterial profile as at baseline, whereas the bacterial profile in those who had stopped smoking was divergent.[57]

Smoking cessation in patients with periodontitis is beneficial for promoting a health-compatible subgingival microbial community.[58] Together with the clinical outcomes, the beneficial effects of smoking cessation on microbiologic parameters clearly warrant smoking cessation counseling along with periodontal treatment.[59]

2.5 | In vitro studies

Essential metabolic functions of commensal bacteria are significantly influenced by exposure to smoke and the virulence of genes increases in pathogenic bacteria, whereas early and widespread cell death is observed in symbiotic bacteria. Overexpression of various pathogenic pathways can be detected in pathogenic biofilms following exposure to tobacco smoke. The anaerobic environmental conditions favor growth of facultative anaerobic periodontopathogens. Therefore, smokers have pathogen-rich, commensal-poor biofilms in

disease and the dysbiosis is established long before the onset of clinical signs of periodontitis.[43,60] Various in vitro models of periodontal biofilm have investigated the possible effects of exposure to smoke on the microbiome.

In a recent study, the influence of cigarette consumption on short chain fatty acid production by anaerobic oral pathogens was investigated.[61] The selected representative oral pathogens were *Filifactor alocis*, *F. nucleatum*, and *P. gingivalis*, which were exposed to a physiologically relevant dose of cigarette smoke extract. The authors reported that cigarette smoke extract did not affect the growth of these bacterial species and their production of short chain fatty acids exhibition varied greatly. The authors concluded that a reduced production of propionic acid, isobutyric acid, butyric acid, and isovaleric acid might at least partly explain the reduced vascular response to periodontal microbiota in smokers.

Metabolic alterations in oral bacterium, *Capnocytophaga sputigena*, as a result of exposure to smokeless tobacco were assessed and the capability of this bacterium to metabolize nicotine was analyzed in an in vitro study.[62] It was reported that smokeless tobacco extracts caused oxidative stress in the bacterium and the arginine-nitric oxide pathway was perturbed. In another in vitro study, *P. gingivalis* infection decreased cigarette smoke condensate-induced gingival epithelial cell migration, but the relevant mechanisms have yet to be clarified.[63] In an earlier, similar study conducted by the same group, low concentrations of cigarette smoke condensate were shown to increase the invasion of human gingival epithelial cells by *P. gingivalis*.[64] Nicotine is the highly addictive component in tobacco and it is among the most hazardous and active of components. The possible effects of nicotine on the growth of *Streptococcus gordonii*, biofilm formation, and cell aggregation were investigated.[65] Biofilm formation was increased at 0.5-4 mg/mL of nicotine, whereas confocal laser scanning electron microscopy revealed that bacterial cell mass was increased by 2 and 4 mg/mL of nicotine. These effects may lead to later pathogen attachment to tooth surfaces, ease the accumulation of calculus, and increase the susceptibility to developing periodontal disease in smokers. Accordingly, cigarette smoke extract has been suggested to augment *P. gingivalis* biofilm formation by increasing FimA avidity, which, in turn, supports initial interspecies interactions and subsequently promotes biofilm growth.[66] Cigarette smoke extract exposure has also been shown to regulate *P. gingivalis* genes, including detoxification and oxidative stress-related genes, DNA repair genes, and those genes related to virulence.[67] The findings indicate that cigarette smoke extract creates environmental stress and that *P. gingivalis* reacts to this by altering its gene expression and outer membrane proteins.

The major tobacco derivatives, nicotine and cotinine can induce modifications to the virulence factors of *P. gingivalis*. The possible effects of nicotine on the growth and protein expression of *P. gingivalis* were investigated in bacterial cell culture and the authors suggested that nicotine has an inhibitory effect on the growth of *P. gingivalis*, and also has the potential to modulate protein expression.[68] The presence of nicotine during differentiation of dendritic cells has been suggested to modulate the immunoregulatory functions of cells stimulated by the lipopolysaccharide of *P. gingivalis*.[69] The effects of nicotine and cotinine on the proteomic profile of *P. gingivalis* were investigated by Cogo et al[70] using two-dimensional electrophoresis, and an increase in those proteins involved in metabolism, virulence, and acquisition peptides, protein synthesis and folding, transcription, and oxidative stress was detected.

Neutrophil respiratory burst is one of the major defense mechanisms against periodontal pathogens. In a cell culture study, neutrophils from periodontally healthy individuals were treated with cotinine, nicotine, and cigarette smoke extract before stimulation with *F. nucleatum*, IgG-opsonized *Staphylococcus aureus*, and *Escherichia coli* lipopolysaccharide.[71] The findings demonstrated that cigarette smoke extract reduced the ability of neutrophils to generate reactive oxygen species after stimulation with the bacteria. Thus, smoking may differentially affect neutrophil functions, in general preventing elimination of periodontal pathogens and, in heavy smokers, also stimulating the release of reactive oxygen species and oxidative stress-mediated tissue damage. In another study, the oxidative activity of neutrophils was explored when stimulated with *P. gingivalis*, nicotine, or both.[72] The authors reported that, in the presence of *P. gingivalis*, nicotine did not further enhance the release of reactive oxygen species by neutrophils, suggesting that bacteria induced the maximum amount in this model.

In an in vitro model of the microbial-mucosal interface, smoke-treated commensals but not pathogens demonstrated an early and significant loss of viability, suggesting that the effects of smoking on the microbiome substantially contribute to its detrimental effects on host-bacterial interactions.[73]

In vitro studies cannot simulate the exact and complete conditions in the oral cavity or periodontal pocket. Therefore, although they have some potential in clarifying certain key mechanisms, the findings of in vitro studies should be evaluated with caution.

2.6 | Experimental models

Smokeless tobacco products can also have significant detrimental effects on oral and systemic health. The impact of smokeless tobacco on oral microbiota was investigated in vivo in an experimental study using high-throughput sequencing in hamsters.[74] Hamsters were treated with 0, 2.5, 25, or 250 mg of smokeless tobacco products and oral bacterial species were determined by metagenomic sequencing of 16S ribosomal RNA. Significant disruption of oral microbiota was detected, in particular after 4 weeks of treatment with smokeless tobacco products.

2.7 | Other tobacco products

There are limited studies of the hazardous effects of tobacco products such as electronic cigarettes or electronic cigarettes on the periodontal microbiome. Electronic cigarettes are an increasingly popular way of using nicotine and the rate of smoking waterpipe

tobacco has been increasing in many countries, particularly among adolescents. Our understanding of how vaping affects oral health is only in its infancy.[75] Oral microbiome profiles were compared among nonsmokers, current smokers, and those who use electronic nicotine delivery systems via 16S amplicon sequencing. The oral microbiome composition differed significantly between smokers (electronic and regular cigarettes) and those who had never smoked, with less *Bacteroidetes* and more *Actinobacteria* in both of the smoking groups compared with the nonsmoking group. Another study by the same group focusing on systemically and periodontally healthy individuals reported 1353 genes unique to individuals using electronic cigarettes and these genes encoded for antibiotic resistance, motility chemotaxis, stress response, horizontal gene transfer, cell wall, iron acquisition, and membrane transport. These functions were mostly attributable to pathogenic species belonging to the genera *Fusobacteria*, *Treponema*, *Prevotella*, and *Bacteroides*.[75] Using the results of these two studies, the authors concluded that the risk of harm associated with electronic nicotine delivery systems to the oral microbiome may be similar to, or even greater than those associated with smoking tobacco. Future prospective and comprehensive studies are warranted to clarify this issue.

3 | PSYCHOLOGICAL STRESS AND PERIODONTAL MICROBIOME

Stress can be described as experiences of physiological or psychological challenges.[76] Exposure to stress factors elevates sympathetic nervous system activity and excites chromaffin cells of the adrenal medulla leading to an increased release of stress hormones, which are called catecholamines. The major catecholamines are epinephrine (adrenaline), norepinephrine (noradrenaline), and dopamine.[77,78] Cortisol is one of the most extensively studied stress-related hormones. Cortisol is a glucocorticoid hormone that is secreted by stimulation of the hypothalamus-pituitary-adrenal axis in response to psychological stress. Cortisol displays circadian rhythms and has been shown to be positively associated with the severity of periodontal disease, systemic disease, age, gender, lifestyle choices such as smoking, and stress.[79] These hormones have important effects on almost all tissues in the human body including cardiovascular, metabolic, endocrine, neuronal, intestinal barrier, and immune systems.[77] Chronic stress induces a shift from T helper 1-linked cellular immunity towards T helper 2-linked humoral immunity and changes the course of infection.[80] In 1992, the concept of microbial endocrinology was proposed, suggesting a bidirectional relationship between microorganisms and human neuroendocrine factors.[81] This theory states that several bacteria use hormones produced by the host to promote bacterial growth and infectious diseases.[82]

Psychological stress can directly affect periodontal health by various biologic mechanisms and it can also have indirect effects through lifestyle changes such as ignoring oral hygiene measures, smoking more heavily, or consuming more fat and sugar.[79] Adults experiencing financial pressure and exhibiting poor coping behaviors were reported to be at an increased risk of severe periodontitis.[82] Patients with periodontitis with inadequate stress behavior strategies (ie, defensive coping) were suggested to be at a higher risk of severe periodontal diseases.[83] In a systematic review, a positive association between stress and periodontal diseases was found in more than half of the studies conducted to date.[84] Salivary and circulating levels of cortisol or hydrocortisone have been reported to increase with the severity of periodontal disease.[85,86]

Possible effects of catecholamines such as noradrenaline on periodontal pathogens have been investigated in numerous studies. It has been demonstrated that this hormone has different effects on the growth of different bacterial species.[87] Noradrenalin reduces the growth of *P. gingivalis* and *A. actinomycetemcomitans* but increases the growth of other species such as *Eikenella corrodens*.[87,88] Interestingly, the same hormone increases the expression of virulence factors like gingipaïns but simultaneously reduces expression of autoinducer,[88] a molecule that is involved in the quorum-sensing process of this bacterium.[89] Adrenaline and noradrenaline can induce changes in gene expression related to oxidative stress and virulence factors in *P. gingivalis*.[90] The possible mechanisms of adrenaline and noradrenaline have been investigated in 43 microbial organisms and it was concluded that autoinducer rather than siderophore-like mechanisms were likely to play an important role in the response of oral microorganisms to stress hormones, contributing to the clinical course of stress-associated periodontal diseases.[91]

Anaerobic microorganisms also respond to chronic psychological stress. Obligate anaerobes such as *P. gingivalis* and *P. intermedia* play an important role in the development of periodontal diseases. Both epinephrine and norepinephrine produced a reduction of ~ 40% in the growth of *P. gingivalis* and the growth inhibition increased dose-dependently with catecholamine concentrations.[92] However, these results are controversial as no inhibitory effect of epinephrine on the growth of *P. gingivalis*, *P. intermedia*, and *F. nucleatum*,[93] no growth effect on *P. gingivalis*, but growth inhibition on *P. intermedia*, *T. forsythia*, and enhanced growth of *F. nucleatum* by both epinephrine and norepinephrine have been documented.[94] A decrease in *F. nucleatum* growth and no growth changes in *P. gingivalis* and *P. intermedia* have also been reported.[95] Positive growth effects of norepinephrine have been found on *Actinomyces naeslundii* and *Campylobacter gracilis* together with inhibitory effects on *P. gingivalis* and *T. forsythia*,[96] and the effects of epinephrine were mostly comparable. Another study failed to find any increased growth of *P. gingivalis* and *Bacteroides fragilis* by supplementation of cultures by epinephrine or norepinephrine.[97]

On the other hand, there are a few studies investigating the effects of dopamine on periodontal microbiome. *Fusobacterium nucleatum* growth was enhanced by dopamine, whereas the growth of *P. gingivalis* was unaffected.[94] The effect of exposure to cortisol, independent of the concentration used, significantly increased the growth of *P. gingivalis* over the first 24 hours, peaking after 12 hours.[98] The authors concluded that these findings

provide further support for the idea that cortisol may influence the growth of *P. gingivalis*. This specific effect may be involved in the relationship between stress and periodontal disease. Accordingly, the impact of cortisol on the community-wide transcriptome of the oral microbiome has been recently investigated and shifts in the gene expression profiles of the oral microbiota have been reported in a similar manner to those observed in vivo during periodontitis.[99] The authors found that pure cultures of *F. nucleatum* showed an increase in biologic processes associated with proteolysis, cobalamin biosynthesis, and iron transport, all of which are related to the progression of periodontitis. An association of *P. gingivalis* with high levels of cortisol was investigated in patients with chronic periodontitis, and serum cortisol levels measured using an immunoassay method showed a significant positive correlation with *P. gingivalis*, but not with *T. forsythia*, *T. denticola*, and *A. actinomycetemcomitans*.[100]

The discrepancies between the findings of various studies may be explained by differences in laboratory methodology and the concentrations of the stress hormones applied. Moreover, different strains of the same species may respond differently to the stress hormones. Another likely explanation may be that when targeting only one strain, as in most of these studies, bacteria may interact with each other, thus changing the outcomes.

It can be stated that epinephrine and norepinephrine mainly affect bacterial gene expression, iron acquisition, and growth, changing their virulence factors and biofilms as a whole. Microbial endocrinology is a novel research area combining two medical disciplines, microbiology and neurobiology. The effects of catecholamines on bacteria, either increasing or decreasing their biologic functions, may have positive or negative outcomes from the clinical point of view depending on the specific situation, as well as the specific bacterial strain. It is clear that changes in biofilm composition and behavior, enhanced bacterial iron acquisition, as well as changes in bacterial gene expression, may sharply increase bacterial virulence, involving bacterial adherence, the infection dose, enzyme production, and the spread and severity of infections.[101,102] Periodontal destruction is the result of an imbalance between bacterial aggression and host response.

It is a fact that hormones have some effects on the metabolism of bacteria, but the mechanisms of crosstalk have still to be elucidated. Furthermore, human hormones can be used by microorganisms as signals to sense changes in their environment, eventually modifying the expression profile to adapt to new conditions. Stress-related hormones are likely to favor the infection by increasing bacterial growth, thereby inducing a breakdown in oral biofilms, but specific mechanisms underlying these effects on periodontal microbiota remain largely unknown and further studies are required to evaluate the possible effects of these hormones, especially on the triggering of virulence factors or quorum-sensing development. Novel research using methodologies such as transcriptomic analysis are warranted for the understanding of the precise interactions between the periodontal microbiome and stress hormones.

4 | DIET AND PERIODONTAL MICROBIOME

Diet type and dietary content are closely related to the oral microbiome. Alterations in dietary macronutrients can lead to a shift of the oral microbiome.[103] Nutrients from human diets such as sugars, fats, and vitamins are also vital nutrients for microorganisms. Saturated fatty acids and vitamin C intake are consistently correlated with alpha (within-subject) diversity indexes in both richness and diversity.[104] The rate of Fusobacteria increases with increasing saturated fatty acid intake. Dietary carbohydrate consumption is important for carious lesions in teeth; however, diet can also affect the development of periodontal diseases. Studies investigating possible modulation by diet have mainly focused on a pathologic perspective and the effects on oral microbiome remain largely obscure.[9] Dietary intake directly affects endogenous nutrients in the oral cavity through systemic circulation.[104] Possible relationships between microbial diversity in saliva and specific diet types such as carnivore, omnivore, and vegan were investigated in 161 individuals but none were found.[105] Most of the published studies aim to clarify the relationship between dental caries and diet but to date few studies have investigated the possible effects of diet on periodontal microbiota. During the last decade, several studies have been published evaluating the possible clinical benefits of probiotics and prebiotics, and some of those studies also assessed microbiologic and biochemical parameters. However, those dietary supplements are beyond the scope of this review.

Alcohol intake was positively associated with periodontal antibodies of the orange-red cluster in serum and inversely associated with the yellow-orange cluster after multivariable adjustment.[106] In a short-term clinical study, 66 volunteers used a mouthwash containing honey for 4 days and effective inhibition of oral bacteria was reported.[107] An alcoholic extract of banana peel was shown to exhibit antimicrobial activity against *P. gingivalis* and *A. actinomycetemcomitans* in an in vitro study.[108]

The interaction between diet and periodontal health has mainly been investigated in terms of clinical periodontal parameters and very few microbiologic studies are available. At present, the available evidence is too sparse and it is too early to form an opinion on whether diet has modulating effects on the oral microbiome.

5 | LIMITATIONS

The current review aims to provide up-to-date data on the effects of environmental factors comprising tobacco products, psychological stress, and diet on the periodontal microbiome. There are some drawbacks to the available studies, such as low sample size and low coverage of sequence. Furthermore, chemical validation of smoking status is of the utmost importance in studies focusing on the possible effects of smoking on clinical, microbiologic, and biochemical parameters. However, some studies rely solely on self-reports when grouping the study participants into different categories of smoker

and nonsmoker. Moreover, being a smoker is regarded as an exclusion criterion in a vast number of studies, particularly those focusing on the clinical outcomes of a particular periodontal intervention. This exclusion approach causes a significant loss of data and evidence. The specific laboratory analysis technique is also very important for the sensitivity and accuracy of findings and this may not be optimal in all of the available studies. Moreover, variations between studies in methodology, inclusion and exclusion criteria for the study participants, and data handling do not enable combining results in order to formulate a consensus.

Possible confounders such as lifestyle, precise oral hygiene habits, alcohol consumption, and drug abuse, as well as the chemical and physical properties of the biologic sample, can influence microbiologic findings.

Advances in omics technologies and improved database clarification and bioinformatics have the potential to identify active microbial networks and the products of interactions. The polymicrobial community in periodontitis exhibits sophisticated structural and functional integration, and further proteomic and metabolomics studies may help to fully unravel the xenobiotic metabolism and its effects on the interrelationships among bacteria of the salivary microbiome.

6 | CONCLUSIONS

Over the past decade, a wealth of data from microbiomic profiling studies has led to the identification of additional molecular drivers in periodontal diseases, however, a limited number of studies have been published focusing on the effects of environmental factors within this context. Omics studies comparing the microbiome in smokers and nonsmokers can unravel markers of resilience. There are exciting opportunities to make improvements in the periodontal health of patients via advances in the understanding of omics. However, microbiome-focused studies should endeavor to include measurements of factors believed to exert a selective effect on microbial communities, such as pH and redox potential, immunologic features, and salivary or gingival crevicular fluid flow rates.[109] These parameters are required to understand the high-dimensional data generated by omics technologies, including RNA sequencing, metagenomics, 16S ribosomal RNA gene profiling, and metabolomics.

High-throughput nucleic acid sequencing technologies have enabled us to examine the taxonomic distribution of microbial communities in periodontal health and disease.[110] Single-marker gene studies assaying bacterial diversity using small subunit ribosomal RNA gene sequences (ie, 16S ribosomal RNA) have revealed highly complex and distinct periodontal disease communities associated with healthy and diseased pockets. Such data for environmental factors are in current demand.

Metagenomic methods provide a less biased assessment of species diversity[111] and can be used to investigate the gene context of microbial communities in relation to disease status and to also provide deeper insight into our understanding of periodontal disease. The combination of 16S ribosomal RNA marker gene sequencing and shotgun metagenomics complement each other in analyses of taxonomic diversity. Those microbes, which possibly eluded examination using earlier investigation techniques, could potentially be captured with omics technology. Metagenomic and metatranscriptomic analyses treat the microbial community as a whole and are not limited to analysis of a predetermined assembly of bacteria. However, these techniques require complete genomes to be performed. Fortunately, the oral community is one of the best characterized in human microbiome, thus facilitating metatranscriptome analysis. The effects of diverse environmental conditions such as exposure to tobacco smoke or stress upon the oral community can be better understood using these techniques. On the other hand, the limited amount of biomass appears to be a technical problem. Pooling samples is one strategy that can overcome this.[112]

Today, we are at the stage of generating some conclusions associated with the functional profiles of the microbiome in health and disease. The first conclusion is that, despite high interpatient variability in microbiome composition, disease-associated communities displayed conserved functional activities, while health-associated communities exhibited a more diverse range of functional activities.[112,113] With metatranscriptomic analysis, it is possible to closely examine specific members of the microbiome to assess their activities.[114,115]

7 | FUTURE CONSIDERATIONS

Untreated severe periodontitis may cause tooth loss, and impaired oral health decreases an individual's quality of life and self-esteem. Tobacco smoking continues to be a major environmental risk factor for periodontitis. As dentists, we should be aware of the great power we possess to help our patients stop smoking. We should train students to increase their skills and knowledge in smoking cessation counseling. We need more evidence of the effects of smoking on oral health. Therefore, we should include smokers in intervention studies and compare their data with those of nonsmokers, rather than excluding them. We should be more attached to the principles of nonsurgical periodontal treatment to treat periodontal diseases in smokers. Psychological stress is another major environmental risk factor that can be controlled, and merits further consideration because existing data show a close interaction with periodontal microbiome. Future studies investigating a possible relationship between diet and periodontal microbiome are warranted.

Longitudinal studies may help to identify the real actors in the onset and progression of periodontal disease in smokers or in those under psychological stress. Future improvements in proteomics and metabolomics may enable determination of the origin of proteins and metabolites produced by the microbiome under different environmental conditions. In these studies it is important to obtain high-quality phenotypes, to apply strict criteria for determining what is a significant signal or variant, and to ensure target biomaterial of both sufficient quantity and quality.

Macro- and microorganisms living in the same environment have multiple influences and complex interactions occurring amongst them. Omics technologies will help with understanding the overall picture comprising environmental factors, microorganisms, and periodontal disease.

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