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***Review Article***

# *Porphyromonas Gingivalis* in the Development of Periodontitis: Impact on Dysbiosis and Inflammation

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**Abstract**

Chronic periodontitis is an inflammatory disease of the dental plaque and affects the soft tissues supporting the tooth. It is one of the most practical oral health issues across the globe and adversely affects the quality of life. In a neutrophil-mediated action, the inflammatory response to periodontitis destroys the periodontal ligaments, gums, the alveolar bone, and the cementum. Some of the most associated invasive pathogens with periodontitis are *Porphyromonas gingivalis*, *Aggregatibacter actinomycetecomitans*, and *Fusobacterium nucleatum*. Google Scholar and PubMed were used to search the evidence using key terms like 'periodontitis,' '*Porphyromonas gingivalis*,' 'Oral Dysbiosis and Periodontitis,' '*Porphyromonas gingivalis* and Periodontitis,' etc. Only studies were included reviewing the *Porphyromonas gingivalis* and its role in periodontitis. It has been observed from several oral pathogens that *P. gingivalis* has received immense attention due to a strong association between *Porphyromonas gingivalis* and periodontal disease. *Porphyromonas gingivalis* also disrupts the delicate balance between various members of the oral microbial communities and promotes oral dysbiosis. The dysbiotic state of the oral microbiome is distinct in functional capabilities and shows a higher expression of genes involved in lipopolysaccharide synthesis, energy regulation, and bacterial motility. Certain virulence factors such as gingipains, LPS, and fimbriae also increase the invasion and pathogenicity of *Porphyromonas gingivalis*. Its presence in the periodontal tissues increases the secretion of numerous pro-inflammatory mediators such as TNF-α, IL-8, and IL-1β, leading to the destruction of soft gingival tissues and ligaments. Early detection of periodontitis and immediate treatment can prevent soft tissue destruction and dentition loss. In conclusion, details about the oral microbiome, oral dysbiosis, and inflammation may offer new therapeutic options in the future, including a personalized approach and the use of combination therapy.

**Keywords:** Periodontitis, Oral microbiota, Oral Dysbiosis, *Porphyromonas gingivalis*, Periodontitis Treatment

## 1. Context

The two most common global oral health issues are periodontal disease, including gingivitis and periodontitis. Both diseases destroy soft tissues that support the teeth. Gingivitis is a medical condition characterized by inflamed gums, bleeding, and chronic pain. If left untreated, gingivitis subsequently progresses to a severe form called periodontitis. The Global Burden of Disease Study (2016) revealed that periodontal diseases were the world's 11$^{th}$ most common medical condition (1).

The overall prevalence rate of periodontal diseases is also very high, with an estimated 20%-50% of the population worldwide affected by the disease (2). As mentioned, periodontal diseases are complex oral diseases characterized by a loss of tooth-supporting tissue. The primary causative factor for periodontal diseases is bacteria-induced inflammation, which can

weaken bones, cause tooth loss, and cause deterioration of soft tissues such as gums. However, the intensity and progression of periodontal diseases largely depend on the type of bacteria involved in the pathogenesis (3). During the advanced stages, the chronic inflammatory condition in the periodontium causes loss of ligaments and, subsequently, loss of alveolar bone and the surrounding tissues (4). Some of the most common risk factors for periodontal disease are smoking, poor oral hygiene, and diabetes, suggesting the multifactorial nature of the disease. Irregular use of toothbrushes and improper toothbrushing techniques can lead to dental plaque accumulation and promote gingival inflammation. This gingival inflammation is the causative factor for the loss of periodontal tissues (5).

Although chronic periodontitis generally affects adults, a severe form of periodontitis is occasionally observed in children. During the initial stages, dysbiosis of oral microbiota leads to the formation of dental plaque, and the interaction with the host immune system promotes inflammation. These pathophysiological changes associated with the disease persist until the inflammation subsides using therapeutic interventions such as biofilm removal. The most commonly observed therapeutic interventions are antibiotics, laser therapy, tissue regeneration, and host modulation therapy (6). The use of antimicrobial helps in reducing the levels of anaerobic infectious agents such as *P. gingivalis*, *Bacteroides forsythus*, and *Treponema denticola* in the oral cavity/dental plaque (7). It is important to mention that a close association between periodontal disease and other systemic diseases exists. Individuals with periodontitis are at risk for kidney ailments, cardiovascular disorders, obesity, rheumatoid arthritis, cognitive impairment, cancer, and respiratory disorders.

It has been hypothesized that periodontitis facilitates the entry of pathogenic periodontal bacteria into the systemic circulation via the damaged epithelium lining of the periodontal pockets. Alternatively, the intense inflammatory response against periodontal bacteria can trigger systemic inflammation and damage body organs (8). The presence of periodontitis is also a predictor of the death rate associated with ischemic heart disease and diabetic nephropathy. It has been observed that cardio-renal mortality is 3.2 times higher in subjects with severe periodontitis than in the control group with no or mild periodontitis (9).

The present review attempts to understand the highly complex etiology of periodontitis and the role of *P. gingivalis* as a central player in the onset and subsequent progression of periodontitis.

## 2. Evidence Acquisition

Different keywords were used to find relevant research studies using two widely used databases, i.e., Google Scholar and PubMed. Keywords used to search the relevant studies were, 'periodontitis', '*Porphyromonas gingivalis*', 'oral dysbiosis', '*Porphyromonas gingivalis* and periodontitis', 'Periodontal dysbiosis and systemic disorders', 'periodontitis and therapeutic strategies", etc.

Inclusion and exclusion criteria were used to select only articles that reviewed how *P. gingivalis* causes periodontitis and inflammation and what possible treatment options could be. All other studies were excluded as they were not up to the inclusion criteria of this study. Besides the direct search, cross-referencing was also employed from the already reviewed and included studies to extend the search for more relevant articles. Only published articles were included in this review, and all other articles and studies were excluded.

## 3. Results

### 3.1. Periodontitis: Prevalence, Causes, and Diagnosis

The incidence and prevalence of periodontal disease are associated with overall socioeconomic status, including the family income and education level. For example, poor individuals living close to a disadvantaged neighborhood are more prone to developing periodontitis (10). In another study, poor oral hygiene, sociodemographic factors, and malocclusion are significant predictors of periodontal

disease in the adult population (11). The prevalence of chronic periodontitis is influenced by age, with the senior population at the highest risk for developing chronic periodontitis (82%). The prevalence in the adolescent and adult populations was 59% and 73%, respectively. Age factor also influences the severity of the periodontal disease. For instance, late adolescence (35%) show gingival disease, the early elderly group (23%) develops chronic periodontitis, while the late adult group (33%) develops an aggressive form of periodontitis (12). Poor oral health is also a major causative factor for the development of periodontitis. It has been observed that fair and poor oral health can increase the risk for periodontitis twice, respectively, compared to individuals with good oral health.

Moreover, good tooth brushing practice and regular visits to the dentist can significantly reduce the risk of periodontitis (34% and 32%, respectively) (13). The World Health Organization has recommended the Community Periodontal Index or CPI to assess the severity and degree of periodontal disorders. The three significant features for calculating CPI are dental calculus, bleeding, and gingival sulcus. On a CPI scale, periodontal diseases are categorized into 5 categories based on the severity associated with the disease. On the CPI scale, a value of zero (0) indicates healthy gums and periodontium devoid of any signs of inflammation. In contrast, a value of 4 indicates severe damage to gums and a loss of teeth. Moreover, a lower CPI value also indicates the success rate and type of treatment strategy needed to cure the disease.

For instance, periodontal diseases of degree 1 can be easily cured by improving oral hygiene, and periodontal diseases of degrees 2 and 3 require treatment and care by a dentist. However, periodontal diseases of degree 4 need the highest level of care and may need surgical interventions (14). Periodontitis is classified into 4 stages based on inflammation and tissue damage. Stage I periodontitis indicates an early stage of disease development and shows attachment loss. In this stage, chronic inflammation and biofilm dysbiosis are the significant factors for the development of periodontitis. Stage II periodontitis is an established state of periodontitis, and careful teeth examination can identify some of the damages caused by disease progression. Stage III periodontitis indicates the advancement of the disease, and significant damage to soft periodontal tissue happens in this stage. Without advanced treatment, stage III periodontitis can lead to tooth loss. In the most advanced stage IV, a significant loss of periodontal support tissue is observed. If left untreated, the progression of the disease can lead to loss of masticatory function, and a patient may lose dentition (15). The initial clinical diagnosis of periodontitis starts with visual observation. A dentist usually observes oral hygiene (plaque and calculus) and gingival inflammation (redness, change in the color of soft tissues, swelling). After initial visual examination, probing depth is assessed using a probe that helps understand the status of inflammation, *i.e.,* the probe reaches the junctional epithelium if inflammation is present and does not reach the junctional epithelium in the absence of inflammation. Radiographic assessment is required to assess the extent and patent of alveolar bone loss due to periodontitis. However, radiographic assessment is secondary to the visual examination, and its use is generally minimized to control radiation dose (16).

### 3.2. Correlation between Oral Dysbiosis and Periodontitis

Periodontitis is characterized by the formation of a pathogenic biofilm around the teeth. The biofilm formation is triggered by accumulating several bacterial species around the teeth, interacting cooperatively and competitively to establish a pathogenic biofilm at the soft gingival sites. At this site, a host immune response to eliminate pathogens damages the host by causing alveolar bone loss (17). Chronic periodontitis affects ~65 million adults in the United States annually. Oral dysbiosis and change in the composition of oral microbiota are often cited as causative factors for the development of chronic periodontitis. A case-control

study has demonstrated that patients with periodontitis show reduced microbial diversity and altered microbiota composition. A higher proportion of *Fusobacterium* and *Porphyromonas* were associated with periodontitis, while healthy individuals show a higher proportion of *Rothia* and *Streptococcus*. Interestingly, the microbiota of periodontitis patients also displayed altered functional capabilities. For instance, the expression of genes involved in energy metabolism, lipopolysaccharide synthesis, and bacterial motility increased in the periodontitis microbiome. Moreover, the microbiome associated with healthy subjects showed increased expression of genes involved in transporters, amino acid biosynthesis, glycolysis/gluconeogenesis, and certain transcription factors (18). Oral microbiota dysbiosis induced by *P. gingivalis* triggers the periodontal disease. It has been observed that colonization by *P. gingivalis,* even at lower levels, can disrupt the homeostatic balance of normal oral microbial communities leading to inflammation and bone loss. Interestingly, both commensal microbiota and complement system is needed for *P. gingivalis*-induced bone loss because germ-free mice and mice deficient in C3a and C5a receptors do not develop bone loss when inoculated by *P. gingivalis.* These data show the crucial role of low-abundance bacterial species in disrupting the homeostatic balance between the host and oral microbiome. The experimental results can help find novel therapeutic targets for treating inflammatory disorders caused by low-abundance bacterial species (19). A recent study in 23 adults with periodontitis demonstrated that *Actinomyces* species were the most found microbial community in both supra and subgingival plaque patients. However, the supragingival surface showed a higher level of *Actinomyces, Streptococcus, Capnocytophaga,* and *Neisseria* species than the subgingival surface from the same tooth. Furthermore, subgingival samples showed an increased proportion of "red" and "orange" complex species, while the supragingival surface had a higher proportion of "green" and "purple" complex species (20). In another observation, *P. gingivalis* and *T. forsythia* were more frequently detected in active periodontitis subjects (21). Periodontal pathogens *P. gingivalis* and *T. forsythia* showed increased expression of genes associated with proteases, peptidases, iron transport, Ton-B receptors, hemolysin, CRISPR-associated genes, and genes associated with aerotolerance. Importantly, several bacterial species that are non-pathogenic under normal circumstances (*Streptococcus, Pseudomonas fluorenscens, and* Veillonella *parvula*) also displayed increased expression of virulence factors. Interestingly, increased expression of genes involved in potassium transport, cobalamin synthesis, and proteolysis was associated with the progression of the disease. The study highlighted the importance of various metabolic signatures in advancing periodontal disease. It demonstrated that the severity of the disease is contributed by several microbial species of the oral microbiome and is not limited to just a few microbes (22). Deng, Szafrański (23) reported that patients with chronic periodontitis show increased activity of red-complex pathogens such as *Prevotella intermedia*, *Filifactor alocis*, *Fretibacterium fastidiosum*, and *Selenomonas sputigena*. The upregulation of arginine deiminase arcA from *P. gingivalis* and *Mycoplasma arginini* was also reported in these subjects. Interestingly, protozoan *Entamoeba gingivalis* was also a dominant species in periodontitis. Further, oral dysbiosis in periodontitis also altered the gene expression profile in the human host, with patients showing significant enrichment of genes associated with ferric iron-binding protein and cancer genes like nucleolar phosphoprotein B23, beta-enolase, and ankyrin repeat domain 30B-like protein. The study established that oral dysbiosis can impact the host's normal gene expression profile and adversely affect human health (23). New findings have suggested that oral dysbiosis caused by *P. gingivalis* is strain-dependent. For instance, *P. gingivalis* strain W83 caused periodontitis while TDC-60 only caused moderate lesions in the C57BL/6 mice model. Mice

treated with virulent *P. gingivalis* strain W83 can cause oral dysbiosis and display lower species richness and evenness than the healthy controls (24). Subjects with periodontitis showed an increased number of periodontitis-associated microbiota as reflected by the concentration of beta-diversity at the genus level. Healthy subjects showed a higher prevalence of *Neisseria, Rothia, Actinomyces Veillonella,* and *Corynebacterium* genera, while *Treponema, Eubacterium, Tannerella, and Campylobacter* genera were higher in chronic periodontitis (25). Studies in mice models have demonstrated that transferring the dysbiotic oral microbiome from a diseased animal to a healthy animal can successfully establish a dysbiotic oral microbial community in the healthy animal. Although treatment with antibiotics reversed oral dysbiosis, a cessation of antibiotics reestablished the dysbiotic oral microbiome suggesting the resilient nature of dysbiotic oral microbiota. The data indicate that the dysbiotic community is stable and can transmit the infection similar to the other conventional infectious agents (26).

### 3.3. *Porphyromonas gingivalis* and Periodontitis

*P. gingivalis* is one of the most extensively studied pathogenic bacteria for periodontitis. *P. gingivalis* is an anaerobic, gram-negative bacterium with a unique ability to modulate the immune system. *P. gingivalis* is often considered a main etiological factor in the progression of periodontitis because it secretes several virulence factors (LPS) and extracellular proteases (gingipain), which destroy soft tissues surrounding the teeth (3). In a Spanish population, periodontitis patients show a significantly higher prevalence of *P. gingivalis* genotypes II and Ib than healthy or gingivitis subjects. The study found a strong correlation between chronic periodontitis and *P. gingivalis* genotypes II, and genotypes II was the most commonly observed in periodontitis patients (27).Virulence factors (LPS, fimbriae, proteases) secreted by *P. gingivalis* facilitate the formation of dental biofilm by promoting coaggregation of *P. gingivalis* with other bacterial communities. Moreover, these factors facilitate bacterial colonization and the growth of a bacterial community. The virulence factors also modulate the functions of the immune system and prevent the clearance of pathogenic microbes by the immune system or promote an inflammatory milieu in the surroundings (28). It has been demonstrated that fimbriae of *P. gingivalis* can strongly interact with several host proteins such as fibrinogen, hemoglobin, and various salivary components. These interactions of fimbriae with an array of host proteins facilitate the adhesion of *P. gingivalis* in the oral cavity and increase the pathogenic character of *P.* gingivalis (29). Interestingly, bacterial species facilitate the attachment of *P. gingivalis* to human fibroblast. For instance, the attachment of *P. gingivalis* to human fibroblast increased up to 10-times in the presence of *Fusobacterium nucleatum.* Sugars like galactose, fucose, and lactose inhibited this higher attachment of *P. gingivalis*. The study demonstrated that bacterial species like *F. nucleatum* are primary colonizers of the host tissue. The common occurrence of *P. gingivalis* and *F. nucleatum* at the endodontic sites is advantageous for *P. gingivalis* growth and growth pathogenesis (30). The co-occurrence of *F. nucleatum* and other *Porphyromonas* strains also facilitates the coaggregation of these strains and promotes the formation of bacterial biofilms (31). Studies have demonstrated that invasion of gingival epithelial cells by *P. gingivalis* is an active process and requires the energy of both *P. gingivalis* and epithelial cells. The invasion also depended on the growth phase of *P. gingivalis*, with lower invasion observed in the lag phase. Moreover, *P. gingivalis* demonstrated higher invasion at low multiplicity, and the invasion reached saturation at higher multiplicity. Furthermore, the invasion was inhibited in the presence of microfilaments and microtubule inhibitors, suggesting a crucial role of epithelial cell cytoskeleton proteins in the invasion process. The inhibitors of *P. gingivalis* proteases inhibited the invasion process

indicating the pivotal role of *P. gingivalis* proteases in the invasion process (32). The pathogenic and virulent nature of *P. gingivalis* is further enhanced by the presence of potent proteases such as gingipains. Gingipains are trypsin-like cysteine proteinases and play several crucial roles, such as uptake of amino acids and maturation of fimbriae. Gingipains (HRgpA and RgpB) also increase vascular permeability and activates the blood coagulation system. Their action at the infection site enhances inflammation and facilitates alveolar bone loss. Kgp is the most powerful gingipains responsible for the bleeding observed in the gingiva sites. Gingipains also help sustain colonization of *P. gingivalis* by degrading the macrophage CD14 leading to inhibition of leukocytes by LPS receptors (33). It has been reported that fimbriae of *P. gingivalis* play an important role in the adhesion and invasion process of gingival epithelial cells. The interaction between fimbriae and β1 integrins of gingival epithelial cells is central to the overall invasion process and subsequent cell response displayed by the cells after *P. gingivalis* infection (34). Inhibition of apoptosis in gingival epithelial cells by *P. gingivalis* is an important adaptation for the survival of *P. gingivalis*. The inhibition of apoptosis in gingival epithelial cells prevents the death of the host cells and facilitates the survival of *P. gingivalis. P. gingivalis* achieves this by inhibiting the activity of caspase 3, a pro-apoptotic enzyme. Of note, the anti-apoptotic nature of *P. gingivalis* is strain-independent and does not depend on the presence of fimbriae (35). Although a large number of *P. gingivalis* grows inside the gingival epithelial cells, the cells remain viable. Infection with *P. gingivalis* increased cell survival by activating PI3K/Akt pathway, inhibiting mitochondrial transmembrane depolarization, and blocking the cytochrome C release (36). Boisvert and Duncan (37) reported that gingipains facilitate host-bacterium interactions. Moreover, gingipain adhesin peptide A44 promotes the entry and internalization of bacteria inside the epithelial cells by hijacking the clathrin-dependent endocytosis system of the cells. Furthermore, the peptide enters into mitochondria and prevents staurosporine-induced apoptosis by upregulating the expression of key anti-apoptotic genes such as *bcl-2* and *bcl-XL*. This anti-apoptotic nature prevents the clearance of *P. gingivalis* by the host. It benefits the bacteria by increasing its survival in the protected cellular environment and aiding in the overall pathogenesis of the bacterium (37). Stathopoulou, Galicia (38) demonstrated that gingival epithelial cells infected by *P. gingivalis* showed increased apoptosis mediated by the gingipains-dependent pathway. Cells infected with *P. gingivalis* showed higher expression of pro-apoptotic molecules such as caspase-3,-8,-9, Bid, and Bax 24 hrs after infection. Although the levels of anti-apoptotic Bcl-2 were upregulated, they failed to inhibit apoptosis. The time and dose-dependent apoptosis were significant between 12-24 hrs of infection. The contrasting results indicate that the dose and duration of *P. gingivalis* may play a critical role in deciding whether *P. gingivalis* will induce apoptosis or inhibit it (38). Bugueno, Batool (39) developed a 3D spheroid model of gingival epithelial cells (EC) and oral fibroblasts (FBs) in a novel experiment. The developed 3D spheroids showed a well-organized structure with epithelial cells forming the external layer while the fibroblasts were at the core. It was observed that *P. gingivalis* reached the core (fibroblast layer) after bypassing the external epithelial cell layer and induced apoptosis at the fibroblastic core. The study has important implications for understanding the interactions between EC-FB and may aid in developing novel therapeutic molecules (39). Recently, the role of ATP-binding cassette (ABC) transporter genes in the pathogenicity of *P. gingivalis* has been deciphered. Gao, Ma (40) reported that expression levels of 2 ABC transporter genes PG_RS04465 (PG1010) and PG_RS07320 (PG1665) were significantly increased in *P. gingivalis* during the co-culturing process with gingival epithelial cells. Moreover, knock-out mice of PG_RS07320 reduced the endotoxin levels in *P. gingivalis*. Both mutant strains displayed a lower activity of gingipains activities and significantly

reduced both adhesion and invasion. The secretion of inflammatory cytokines interleukin-1β (IL-1β) and IL-6 was also reduced in knock-out strains. Furthermore, mice infected with knock-out strains showed reduced mortality than the wild-type strain suggesting that ABC-transporter genes positively regulate the pathogenicity of *P. gingivalis* and aid in the virulence (40). It has been reported that the potent inflammatory molecule TNF-α augments the invasion capability of *P. gingivalis* in gingival epithelial cells by increasing the expression levels of Intercellular Adhesion Molecule 1 (ICAM-1) and activating Rab5. Antibodies to both TNF- α receptor 1 and ICAM-1 inhibited the invasion of *P. gingivalis* (41). Recent studies have suggested that persistent infection with *P. gingivalis* human immortalized oral epithelial cells caused increased cell proliferation and abnormal morphological changes and promoted cell migration and invasion. This suggests that persistent *P. gingivalis* infection may lead to the development of oral cancer (42).

### 3.4. Periodontal Dysbiosis, Inflammation, and Systemic Disorders

Dental plaque is a well-organized biofilm with defined structural and functional features and contains various polymers of both host and bacterial origin. The formation of dental plaque on teeth surfaces is a natural phenomenon, and it helps maintain healthy oral microbiota by preventing the unwanted colonization of exogenous species (43). The microbial community of the oral cavity is the second-largest and most diverse after the gut microbial community (44). A stable oral microbiota is a consequence of synergistic and antagonistic interactions among the microbiota community, thus making its compositions relatively constant despite constant exposure to various environmental triggers (45). However, plaque formation around the gingival margin elicits an inflammatory response from the host and increases the secretion of gingival crevicular fluid. This process leads to oral dysbiosis and drastically alters the composition of oral microflora from gram-positive to obligate anaerobic gram-negative species. This suggests that disease progression can be treated by reversing periodontal dysbiosis and maintaining microbial homeostasis. According to the "Ecological Plaque Hypothesis," a close association between the plaque microflora and the host can be used to design novel therapeutic strategies to prevent diseases (46). As a homeostatic mechanism, a low-grade "surveillance" inflammation is caused by neutrophils present at the gingival sites and considered normal. However, plaque formation at the gingival sites can cause prolonged inflammation, and the affected person eventually develops gingivitis. This destructive process leads to the loss of collagen at the gingival sites. The loss, however, is reversible if inflammation is resolved. Importantly, unresolved inflammation ultimately leads to periodontitis which makes the loss of ligament and alveolar bone irreversible (47). The development of chronic inflammation at periodontal pockets can significantly alter the nutrient and redox potential and increases the overall microbial diversity and number of species, thus inducing oral dysbiosis. The onset of a dysbiotic state further exacerbates the inflammatory milieu and promotes bone destruction (47). A dysbiotic microbiota harbors keystone pathogens and other pathogenic organisms and survives longer against the immune clearance by displaying a synergistic virulence. Longer sustenance of these microbial communities initiates a self-feeding vicious cycle of inflammation and bone loss, leading to numerous systemic complications (48). The dysbiotic microbial communities utilize the breakdown products of tissue destruction as a nutrient that helps them thrive (49). Certain metabolites secreted by the dysbiotic microbial community can act as a metabolic signature of inflammation and may be used to assess periodontal inflammation. For instance, cadaverine and hydrocinname are associated with increased Periodontal inflamed surface area (PISA), while uric acid and ethanolamine were common in subjects with low PISA. The dental plaque of the high PISA group

showed distinct metabolic signatures, which were reflected in increased activity of butyric acid metabolism, lysine degradation, polyamine metabolism, and arginine and proline metabolism (50). Dysbiotic microbiota further promotes periodontal inflammation by stimulating the expansion of resident memory Th17 cells. The expansion of Th17 cells is dependent on local dysbiosis and requires the presence of cytokines such as IL-6 and IL-23. The accumulation of Th17 cells and neutrophils is one of the prerequisites for initiating tissue inflammation and subsequent destruction of soft tissue in animal models of periodontitis. The study observations suggest that inhibiting Th17 cells can be exploited as a therapeutic strategy to alleviate or reduce periodontal inflammation (51). Recent clinical observations suggest a close association between oral dysbiosis, inflammation, and autoimmune liver disorders (AILD). For instance, patients of autoimmune hepatitis (AIH) showed a higher prevalence of *Veillonella* and a lower abundance of *Streptococcus* in the oral microbiota than the healthy controls. Primary biliary cholangitis (PBC) patients had higher *Eubacterium* and *Veillonella* but lower *Fusobacterium*. Interestingly, an abundance of *Veillonella* caused a significant increase in the levels of pro-inflammatory markers IL-8, IL-1 β, and immunoglobulin A in the salivary samples of AILD patients indicating a close association between salivary inflammatory markers and oral dysbiosis (52). A similar association is observed between oral dysbiosis, inflammation, and Oral squamous cell carcinoma. Patients with OSCC showed a relative abundance of several bacterial species such as *Veillonella parvula*, *Prevotella melanogenic*, *Peptostreptococcus anaerobi*c, and *Fusobacterium* sp. The dysbiosis is associated with increased levels of IFN-γ, IL-6, IL-8, and GM-CSF in the saliva of OSCC patients suggesting that oral dysbiosis may also contribute to increased risk for OSCC due to heightened inflammation (53). A significant association between periodontitis and rheumatoid arthritis (RA) has been observed in human studies. The severity of periodontitis is directly associated with the severity of RA, independent of other factors (54). Periodontal pathogens such as *P. gingivalis* and *Aggregatibacter actinomycetemcomitans* may aggravate the symptoms of RA by increasing the production of RA antibodies by inducing direct post-translational modification of proteins or modulating the functions of neutrophils. Alternatively, dysbiotic oral microbes can reach systemic circulation and cause systemic inflammation and auto-antibodies, thus further worsening RA symptoms (55).

### 3.5. Therapeutic Strategies

Numerous therapeutic strategies have been tested for the treatment of periodontitis. Using mechanical debridement to remove the bacterial biofilms is one of the first steps in combination with antibiotics to treat periodontitis. However, mechanical debridement may not be successful when the infection is caused by invasive pathogens such as *P. gingivalis*, *Actinobacillus actinomycetemcomitans*, or *Prevotella intermedia* (56). The gingival sulcus is home to more than 700 bacterial species, but only a few are involved in disease progression. These findings make the use of antibiotics promising for the treatment of periodontitis (57). The use of systemic antibiotics is beneficial in reinforcing the benefits of mechanical debridement. Systemic antibiotics also reach periodontal tissue and infected sites where mechanical instruments and other topical anti-infective agents do not reach. Further, antibiotics also support the host immune system by killing the pathogens that remained alive after conventional mechanical therapy (58). Azithromycin is a macrolide antibiotic used in the treatment of periodontitis. It concentrates on fibroblasts, macrophages, and neutrophils, cells that directly play a role in the periodontal disease, thus providing sustained protection from periodontal pathogens. Azithromycin also possesses powerful anti-inflammatory and immuno-modulatory properties, thus making it a valuable antibiotic in the management of periodontal diseases (59). Recent studies have, however, found that azithromycin is less effective than a combination of amoxicillin and metronidazole in reducing biofilm

comprised of red-complex bacteria *P. gingivalis*, *Treponema denticola*, and *Tannerella forsythia*. The minimum biofilm inhibitory concentration for azithromycin was 1.6mg/L, while the combination of amoxicillin and metronidazole reduced the biofilm formation to only 1.63mg/L (60). In recent clinical trials, the use of clarithromycin (CLR) as an adjunct therapy to non-surgical periodontal therapy (NSPT) has been successful for shorter periods. Patients in the SRP+CLR group showed significantly lower colony-forming units than the SRP group alone at 3 months. However, no significant difference was observed at 6 months (61). A new therapeutic strategy called "Perioceutics" has emerged in recent times. Perioceutics is the use of host-modulating agents that help in lowering disease severity and accelerate the healing process. Host modulating agents are targeted against Matrix metallopeptidase, lipid-inflammatory mediators, and inflammatory cytokines (62). In periodontitis, the inflammation becomes chronic when the pathogenic bacteria continue to grow, or the host immune system elicits an abnormal and prolonged immune response leading to tissue damage, fibrosis, and unresolved inflammation (63). Thus, inflammation targeting has been suggested as a promising therapeutic strategy to prevent *P. gingivalis*-induced bone loss. Nal-P-113, a novel antimicrobial peptide, protected against *P. gingivalis*-induced bone loss by reducing the numbers of *P. gingivalis and consequently reducing the levels of inflammatory cytokines* IL-1β and TNF-α (64). M101, an oxygen carrier derived from *Arenicola marina*, has shown promising results as an anti-inflammatory and anti-infectious agent. M101 at 1g/L concentration reduced inflammatory mediators such as NF-kB, IL-1β, IL-8, TNF-α, and RANKL in epithelial cells infected with *P. gingivalis*. Also, it increased the expression of pro-healing molecules IL-10, IL-1, platelet-derived growth factor-BB, and transforming growth factor-beta 1. Additionally, the expression of resolvin-E1 and immune modulators TIMP-1and M-CSF also increased. Mice given M101 showed reduced biofilm growth of *P. gingivalis* and lower inflammatory score, suggesting that M-101 can be a novel molecule against *P. gingivalis*-induced periodontitis (65). Treatment with IL-1β/receptor antagonists/antibodies is another proposed therapeutic approach against periodontitis. For instance, Anakinra (Kineret), an FDA- approved drug for inflammatory disorders such as rheumatoid arthritis, may be used in periodontitis. Similarly, recombinant IL-1 antagonist Rilonacept and anti-IL-1β antibody Canakinumab (Ilaris) are other possible candidates against periodontitis (66). Recombinant anti-inflammatory mediators have been proposed to reduce bone loss in periodontitis patients. In a dog model of periodontitis, subcutaneous injection of anti-inflammatory recombinant human interleukin-11 (rhIL-11) significantly reduced periodontal attachment and alveolar bone loss (67). The use of tissue engineering in the treatment of periodontitis has been tested in several studies to regenerate complex periodontal tissue and bones (68). However, periodontal healing is a highly complex process. It involves numerous biochemical and physiological events such as cell proliferation, cell migration, mineralization, and synthesis of several extracellular matrix components. Moreover, the regeneration of alveolar bone, cementum, and ligament becomes challenging due to the involvement of three tissue and intricate interactions among the three tissues (69). For instance, recombinant amelogenin has regenerated lost bone and periodontal ligaments. Treatment with amelogenin for 2 weeks helped regenerate all lost periodontal tissues via recruitment of mesenchymal progenitor cells, which subsequently differentiate into periodontal tissues (70). Recent clinical studies have reported improvement in Oral health-related quality of life (OHRQL), improved bone health, and improved periodontal regeneration when treated with 0.3% recombinant human fibroblast growth factor (rhFGF)-2 (71). Similarly, recombinant human platelet-derived growth factor–BB (rhPDGF) has received USFDA approval for periodontitis and oral regeneration

treatments. It improves bone regeneration and accelerates wound healing (72).

## 4. Conclusion

Periodontitis is a common inflammatory disease of the oral cavity and affects 20-50% of the global population. Studies have established that gram-negative anaerobe *P. gingivalis* is a keystone pathogen in manipulating. A healthy oral microbiome can create a dysbiotic oral microbiota, ultimately causing hallmark inflammation and alveolar bone loss. A close association is observed between periodontitis and other disorders such as rheumatoid arthritis and autoimmune liver disorders. This suggests that reversing dysbiosis and alleviating periodontal inflammation can help reduce tissue destruction and loss of dentition. Some of the commonly employed therapeutic approaches are the use of antibiotics, anti-inflammatory drugs, and use of novel gene therapy approaches. However, details about the oral microbiome, oral dysbiosis, and inflammation are still evolving and may offer new therapeutic options in the future.

## Authors' Contribution

Study concept and design: A. G. B.
Acquisition of data: A. G.
Analysis and interpretation of data: S. N.
Drafting of the manuscript: P. K. M.
Critical revision of the manuscript for important intellectual content: A. G.
Administrative, technical, and material support: A. G.

## Conflict of Interest

The authors declare that they have no conflict of interest.

## References

1. Vos T, Abajobir AA, Abate KH, Abbafati C, Abbas KM, Abd-Allah F, et al. Global, regional, and national incidence, prevalence, and years lived with disability for 328 diseases and injuries for 195 countries, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet. 2017;390(10100):1211-59.
2. Nazir M, Al-Ansari A, Al-Khalifa K, Alhareky M, Gaffar B, Almas K. Global prevalence of periodontal disease and lack of its surveillance. Sci World J. 2020;2020.
3. Rafiei M, Kiani F, Sayehmiri F, Sayehmiri K, Sheikhi A, Azodi MZ. Study of Porphyromonas gingivalis in periodontal diseases: A systematic review and meta-analysis. Med J Islam Repub Iran. 2017;31:62.
4. Nazir MA. Prevalence of periodontal disease, its association with systemic diseases and prevention. Int J Health Sci. 2017;11(2):72.
5. International. JOD. Retracted: Risk Factors of Periodontal Disease: Review of the Literature. Int J Dent. 2021;2021.
6. Kinane DF, Stathopoulou PG, Papapanou PN. Periodontal diseases. Nat Rev Dis Primers. 2017;3(1):1-14.
7. Loesche WJ, Grossman NS. Periodontal disease as a specific, albeit chronic, infection: diagnosis and treatment. Clin Microbiol Rev. 2001;14(4):727-52.
8. Winning L, Linden GJ. Periodontitis and systemic disease. BDJ Team. 2015;2:15163.
9. Saremi A, Nelson RG, Tulloch-Reid M, Hanson RL, Sievers ML, Taylor GW, et al. Periodontal disease and mortality in type 2 diabetes. Diabetes Care. 2005;28(1):27-32.
10. Borrell LN, Beck JD, Heiss G. Socioeconomic disadvantage and periodontal disease: the Dental Atherosclerosis Risk in Communities study. Am J Public Health. 2006;96(2):332-9.
11. Baiju RMP, Peter E, Nayar BR, Varughese JM, Varghese NO. Prevalence and predictors of early periodontal disease among adolescents. J Indian Soc Periodontol. 2019;23(4):356.
12. Tadjoedin FM, Fitri AH, Kuswandani SO, Sulijaya B, Soeroso Y. The correlation between age and periodontal diseases. J Int Dent Medical Res. 2017;10(2):327.
13. Lertpimonchai A, Rattanasiri S, Vallibhakara SA-O, Attia J, Thakkinstian A. The association between oral hygiene and periodontitis: a systematic review and meta-analysis. Int Dent J. 2017;67(6):332-43.
14. Kirch W. CPI (Community Periodontal Index). In: Kirch W, editor. Encyclopedia of Public Health. Dordrecht: Springer Netherlands; 2008. p. 176--7.
15. Tonetti MS, Greenwell H, Kornman KS. Staging and grading of periodontitis: Framework and proposal of a new classification and case definition. J Periodontol. 2018;89:S159-S72.

16. Preshaw PM. Detection and diagnosis of periodontal conditions amenable to prevention. BMC Oral Health. 2015;15(1):1-11.
17. Jiao Y, Hasegawa M, Inohara N. The role of oral pathobionts in dysbiosis during periodontitis development. J Dent Res. 2014;93(6):539-46.
18. Kirst ME, Li EC, Alfant B, Chi Y-Y, Walker C, Magnusson I, et al. Dysbiosis and alterations in predicted functions of the subgingival microbiome in chronic periodontitis. Appl Environ Microbiol. 2015;81(2):783-93.
19. Hajishengallis G, Liang S, Payne MA, Hashim A, Jotwani R, Eskan MA, et al. Low-abundance biofilm species orchestrates inflammatory periodontal disease through the commensal microbiota and complement. Cell Host Microbe. 2011;10(5):497-506.
20. Ximénez-Fyvie LA, Haffajee AD, Socransky SS. Microbial composition of supra-and subgingival plaque in subjects with adult periodontitis. J Clin Periodontol. 2000;27(10):722-32.
21. Tanner AC, Kent Jr R, Kanasi E, Lu SC, Paster BJ, Sonis ST, et al. Clinical characteristics and microbiota of progressing slight chronic periodontitis in adults. J Clin Periodontol. 2007;34(11):917-30.
22. Yost S, Duran-Pinedo AE, Teles R, Krishnan K, Frias-Lopez J. Functional signatures of oral dysbiosis during periodontitis progression revealed by microbial metatranscriptome analysis. Genome Med. 2015;7(1):1-19.
23. Deng Z-L, Szafrański SP, Jarek M, Bhuju S, Wagner-Döbler I. Dysbiosis in chronic periodontitis: key microbial players and interactions with the human host. Sci Rep. 2017;7(1):1-13.
24. Boyer E, Leroyer P, Malherbe L, Fong SB, Loréal O, Bonnaure Mallet M, et al. Oral dysbiosis induced by Porphyromonas gingivalis is strain-dependent in mice. J Oral Microbiol. 2020;12(1):1832837.
25. Meuric V, Le Gall-David S, Boyer E, Acuña-Amador L, Martin B, Fong SB, et al. Signature of microbial dysbiosis in periodontitis. Appl Environ Microbiol. 2017;83(14):e00462-17.
26. Payne M, Hashim A, Alsam A, Joseph S, Aduse-Opoku J, Wade WG, et al. Horizontal and vertical transfer of oral microbial dysbiosis and periodontal disease. J Dent Res. 2019;98(13):1503-10.
27. Puig-Silla M, Dasí-Fernánde F, Montiel-Company J-M, Almerich-Silla J-M. Prevalence of fimA genotypes of Porphyromonas gingivalis and other periodontal bacteria in a Spanish population with chronic periodontitis. Med Oral Patol Oral Cir Bucal. 2012;17(6):1047.
28. Xu W, Zhou W, Wang H, Liang S. Roles of Porphyromonas gingivalis and its virulence factors in periodontitis. Adv Protein Chem Struct Biol. 2020;120:45-84.
29. Amano A, Nakamura T, Kimura S, Morisaki I, Nakagawa I, Kawabata S, et al. Molecular interactions of Porphyromonas gingivalis fimbriae with host proteins: kinetic analyses based on surface plasmon resonance. Infect Immun. 1999;67(5):2399-405.
30. Metzger Z, Blasbalg J, Dotan M, Weiss EI. Enhanced attachment of Porphyromonas gingivalis to human fibroblasts mediated by Fusobacterium nucleatum. J Endod. 2009;35(1):82-5.
31. Okuda T, Kokubu E, Kawana T, Saito A, Okuda K, Ishihara K. Synergy in biofilm formation between Fusobacterium nucleatum and Prevotella species. Anaerobe. 2012;18(1):110-6.
32. Lamont RJ, Chan A, Belton CM, Izutsu KT, Vasel D, Weinberg A. Porphyromonas gingivalis invasion of gingival epithelial cells. Infect Immun. 1995;63(10):3878-85.
33. Imamura T. The role of gingipains in the pathogenesis of periodontal disease. J Periodontol. 2003;74(1):111-8.
34. Yilmaz Ö, Watanabe K, Lamont RJ. Involvement of integrins in fimbriae-mediated binding and invasion by Porphyromonas gingivalis. Cell Microbiol. 2002;4(5):305-14.
35. Mao S, Park Y, Hasegawa Y, Tribble GD, James CE, Handfield M, et al. Intrinsic apoptotic pathways of gingival epithelial cells modulated by Porphyromonas gingivalis. Cell Microbiol. 2007;9(8):1997-2007.
36. Yilmaz Oz, Jungas T, Verbeke P, Ojcius DM. Activation of the phosphatidylinositol 3-kinase/Akt pathway contributes to survival of primary epithelial cells infected with the periodontal pathogen Porphyromonas gingivalis. Infect Immun. 2004;72(7):3743-51.
37. Boisvert H, Duncan MJ. Translocation of Porphyromonas gingivalis gingipain adhesin peptide A44 to host mitochondria prevents apoptosis. Infect Immun. 2010;78(8):3616-24.
38. Stathopoulou PG, Galicia JC, Benakanakere MR, Garcia CA, Potempa J, Kinane DF. Porphyromonas gingivalis induce apoptosis in human gingival epithelial cells through a gingipain-dependent mechanism. BMC Microbiol. 2009;9(1):1-12.
39. Bugueno IM, Batool F, Keller L, Kuchler-Bopp S, Benkirane-Jessel N, Huck O. Porphyromonas gingivalis

bypasses epithelial barrier and modulates fibroblastic inflammatory response in an in vitro 3D spheroid model. Sci Rep. 2018;8(1):1-13.
40. Gao L, Ma Y, Li X, Zhang L, Zhang C, Chen Q, et al. Research on the roles of genes coding ATP-binding cassette transporters in Porphyromonas gingivalis pathogenicity. J Cell Biochem. 2020;121(1):93-102.
41. Kato Y, Hagiwara M, Ishihara Y, Isoda R, Sugiura S, Komatsu T, et al. TNF-α augmented Porphyromonas gingivalis invasion in human gingival epithelial cells through Rab5 and ICAM-1. BMC Microbiol. 2014;14(1):1-13.
42. Geng F, Liu J, Guo Y, Li C, Wang H, Wang H, et al. Persistent exposure to Porphyromonas gingivalis promotes proliferative and invasion capabilities, and tumorigenic properties of human immortalized oral epithelial cells. Front Cell Infect Microbiol. 2017;7:57.
43. Marsh PD, editor Dental plaque as a biofilm and a microbial community–implications for health and disease. BMC Oral health; 2006: BioMed Central.
44. Deo PN, Deshmukh R. Oral microbiome: Unveiling the fundamentals. Journal of oral and maxillofacial pathology: JOMFP. 2019;23(1):122.
45. Sharma N, Bhatia S, Sodhi AS, Batra N. Oral microbiome and health. AIMS microbiology. 2018;4(1):42.
46. Marsh PD. Microbial ecology of dental plaque and its significance in health and disease. Adv Dental Res. 1994;8(2):263-71.
47. Van Dyke TE, Bartold PM, Reynolds EC. The nexus between periodontal inflammation and dysbiosis. Front Immunol. 2020;11:511.
48. Hajishengallis G. Immunomicrobial pathogenesis of periodontitis: keystones, pathobionts, and host response. Trends Immunol. 2014;35(1):3-11.
49. Hajishengallis G. Dysbiosis and inflammation in periodontitis: synergism and implications for treatment. J Oral Microbiol. 2017;9(1):1325198.
50. Sakanaka A, Kuboniwa M, Hashino E, Bamba T, Fukusaki E, Amano A. Distinct signatures of dental plaque metabolic byproducts dictated by periodontal inflammatory status. Sci Rep. 2017;7(1):1-10.
51. Dutzan N, Kajikawa T, Abusleme L, Greenwell-Wild T, Zuazo CE, Ikeuchi T, et al. A dysbiotic microbiome triggers TH17 cells to mediate oral mucosal immunopathology in mice and humans. Sci Transl Med. 2018;10(463).
52. Abe K, Takahashi A, Fujita M, Imaizumi H, Hayashi M, Okai K, et al. Dysbiosis of oral microbiota and its association with salivary immunological biomarkers in autoimmune liver disease. PloS One. 2018;13(7):e0198757.
53. Rai AK, Panda M, Das AK, Rahman T, Das R, Das K, et al. Dysbiosis of salivary microbiome and cytokines influence oral squamous cell carcinoma through inflammation. Arch Microbiol. 2021;203(1):137-52.
54. Rodríguez-Lozano B, González-Febles J, Garnier-Rodríguez JL, Dadlani S, Bustabad-Reyes S, Sanz M, et al. Association between severity of periodontitis and clinical activity in rheumatoid arthritis patients: a case–control study. Arthritis Res Ther. 2019;21(1):1-12.
55. Cheng Z, Meade J, Mankia K, Emery P, Devine DA. Periodontal disease and periodontal bacteria as triggers for rheumatoid arthritis. Best Pract Res Clin Rheumatol. 2017;31(1):19-30.
56. Bogdanovska L, Kukeska S, Popovska M, Petkovska R, Goracinova K. Therapeutic strategies in the treatment of periodontitis. Biol Pharm Bull. 2012;58(1):2.
57. Krayer JW, Leite RS, Kirkwood KL. Non-surgical chemotherapeutic treatment strategies for the management of periodontal diseases. Dent Clin. 2010;54(1):13-33.
58. Kapoor A, Malhotra R, Grover V, Grover D. Systemic antibiotic therapy in periodontics. Dent Res J. 2012;9(5):505.
59. Hirsch R, Deng H, Laohachai M. Azithromycin in periodontal treatment: more than an antibiotic. J Periodontal Res. 2012;47(2):137-48.
60. Ong HS, Oettinger-Barak O, Dashper SG, Darby IB, Tan KH, Reynolds EC. Effect of azithromycin on a red complex polymicrobial biofilm. J Oral Microbiol. 2017;9(1):1339579.
61. Suryaprasanna J, Radhika PL, Karunakar P, Rekharani K, Faizuddin U, Manojkumar MG, et al. Evaluating the effectiveness of clarithromycin as an adjunct to scaling and root planing: A randomized clinical trial. J Indian Soc Periodontol. 2018;22(6):529.
62. Gulati M, Anand V, Govila V, Jain N. Host modulation therapy: An indispensable part of perioceutics. J Indian Soc Periodontol. 2014;18(3):282.
63. Hasturk H, Kantarci A. Activation and resolution of periodontal inflammation and its systemic impact. Periodontology. 2015;69(1):255-73.
64. Wang H-y, Lin L, Fu W, Yu H-Y, Yu N, Tan L-s, et al. Preventive effects of the novel antimicrobial peptide Nal-P-113 in a rat Periodontitis model by limiting the growth of Porphyromonas gingivalis and modulating IL-1β and TNF-α production. BMC Complement Altern Med. 2017;17(1):1-10.

65. Batool F, Stutz C, Petit C, Benkirane-Jessel N, Delpy E, Zal F, et al. A therapeutic oxygen carrier isolated from Arenicola marina decreased P. gingivalis induced inflammation and tissue destruction. Sci Rep. 2020;10(1):1-14.
66. Cheng R, Wu Z, Li M, Shao M, Hu T. Interleukin-1β is a potential therapeutic target for periodontitis: a narrative review. Int J Oral Sci. 2020;12(1):1-9.
67. Martuscelli G, Fiorellini JP, Crohin CC, Howard Howell T. The effect of interleukin-11 on the progression of ligature-induced periodontal disease in the beagle dog. J Periodontol. 2000;71(4):573-8.
68. Fawzy El-Sayed KM, Doerfer CE. Animal models for periodontal tissue engineering: a knowledge-generating process. Tissue Eng Part C. 2017;23(12):900-25.
69. Goker F, Larsson L, Del Fabbro M, Asa'ad F. Gene delivery therapeutics in the treatment of periodontitis and peri-implantitis: a state of the art review. Int J Mol Sci. 2019;20(14):3551.
70. Haze A, Taylor AL, Haegewald S, Leiser Y, Shay B, Rosenfeld E, et al. Regeneration of bone and periodontal ligament induced by recombinant amelogenin after periodontitis. J Cell Mol Med. 2009;13(6):1110-24.
71. Yoshida W, Takeuchi T, Imamura K, Seshima F, Saito A, Tomita S. Treatment of chronic periodontitis with recombinant human fibroblast growth factor-2 and deproteinized bovine bone mineral in wide intrabony defects: 12-month follow-up case series. Bull Tokyo Dent Coll. 2020;61(4):231-41.
72. Tavelli L, Ravidà A, Barootchi S, Chambrone L, Giannobile W. Recombinant human platelet–derived growth factor: A systematic review of clinical findings in oral regenerative procedures. JDR Clin Trans Res. 2021;6(2):161-73.