**Review**

**Oral dysbiotic communities and their implications in systemic diseases**

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**Abstract:** The human body supports the growth of a wide array of microbial communities in various niches, such as the oral cavity, gastro-intestinal and urogenital tracts and on the surface of the skin. These host associated microbial communities include yet-un-cultivable bacteria and are influenced by various factors. Together, these communities of bacteria are referred to as the human microbiome. Human oral microbiome consists of both symbionts and pathobionts. Deviation from symbiosis among the bacterial community leads to “dysbiosis”—a state of community disturbance. Dysbiosis occurs due to many confounding factors that predispose to a shift in the composition and relative abundance of microbial communities. Dysbiotic communities have been a major cause for many microbiomes related systemic infections. Such dysbiosis is directed by certain important pathogens called the “keystone pathogens” that could modulate community microbiome variations. One such persistent infection is oral infection, mainly periodontitis, where a wide array of causal organisms has been implied to systemic infections such as cardiovascular disease, diabetes mellitus, rheumatoid arthritis and Alzheimer’s disease. The keystone pathogens co-occur with many yet-cultivable bacteria and their interactions lead to dysbiosis. This has been the focus of recent research. While immune evasion is one of the major modes that lead to dysbiosis, new processes and new virulence factors of bacteria have been shown to be involved in this important process of that determine disease or health state. This review focuses on such dysbiotic communities, their interactions and their virulence factors that predispose the host to other systemic implications.

**Keywords:** Oral dysbiosis, Human oral microbiome, yet-un cultivable organisms, systemic diseases.

1. **Introduction**

Human microbiome plays a pivotal role in human biology through its influence on many physiological functions such as human development, physiology, immunity and nutrition. Even though the composition of the human microbiome has received considerable attention in recent years, the precise mechanisms whereby these microbial communities mediate disease and restore and maintain health remain unexplored. However, recent studies have shown that several chronic diseases of the mouth and gastro-intestinal tract are associated with alterations in the composition of the microbiome termed as “dysbiosis”. Dysbiosis is a significant harmful shift in the relative abundances and individual components of the microbiome which varies with their composition and relative abundances during health status. This shift causes major dysbiosis related diseases in the humans namely, the periodontitis, irritable bowel syndrome, chronic vaginosis etc. Among them, periodontal disease depicts major dysbiotic condition due to their diversity of genera involved in the normal and periodontal microbiome. Oral microbiota consists of two major types of bacteria namely Gram positive and Gram negative bacteria with more than 700 species of microorganisms found in the oral cavity [1].

The etiology of periodontitis with both Gram positive and Gram negative bacteria suggest a complex heterogeneous microbial population which could be classified as early and late colonizers
The oral cavity also includes several discrete microbial habitats such as gingival sulcus, teeth, attached gingiva, tongue, lip, cheek, hard and soft palate [3]. With a steady transition of various environments such as the oxygen tension and nutrient availability, the bacterial microbiota play a pivotal role in health and disease conditions of which periodontitis is the highly prevalent disease among the world population [4].

The periodontal infections are a distinct group of clinical entities which are caused by bacterial communities developing in a complex polymicrobial synergistic association in oral microbiome[1]. Approximately 47% of adults in the United States aged between 30 years (approximately 65 million adults) have periodontitis: 30.0% with moderate, 8.5% with severe periodontitis and 8.7% with mild periodontitis. Periodontal infections in addition to heavy monetary healthcare burden also have been linked with many systemic diseases [5]. Periodontitis is a chronic inflammatory disease affecting tissues that surround and support the teeth. Its occurrence is associated with important systemic diseases such as cardiovascular disease [6], rheumatoid arthritis [7], and Alzheimer’s disease [8]. One of the most important etiologies of periodontitis is Porphyromonas gingivalis, a keystone Gram negative bacterial pathogen [9]. Keystone pathogens can orchestrate inflammatory disease by remodeling a normally benign microbiota, causing imbalance between normal and pathogenic microbiota (dysbiosis) [9-11]. Dysbiosis of oral microbiome in periodontal disease is a hallmark of this condition. Understanding the mechanism of dysbiosis, its functional relevance to disease and strategies to achieve reversal of dysbiosis to restore health has been the prime focus of research. Recent investigations using the mouse model of this disease have demonstrated that the human periodontal bacterium Porphyromonas gingivalis acts as a keystone pathogen in manipulating the normal commensal microbiome into a dysbiotic condition even when present at low abundance. Furthermore; this dysbiotic microbiome is causative of disease rather than a consequence of the altered environment in this inflammatory condition [10].

In this article, the oral dysbiosis caused due to host-microbiome interaction, and the major mechanisms of dysbiosis and bacterial virulence proteins of co-occurring microbiota that predispose the host to systemic disease are briefly reviewed.

2. Oral microbiota and microbiome

The term “Microbiome” was coined by the Nobel Laureate, Joshua Lederberg. This term was coined to signify the relationship between the micro-organisms and the host such as symbiotic relationship namely commensalism, mutualism and pathogenic. But they are ignored as determinants of health and disease [12]. Oral microbiome otherwise called as oral microflora or oral microbiota is the complex microbial community that resides in the human oral cavity [3]. Approximately 500 to 700 species are estimated to be residing in the oral cavity, of which half of them are cultivated anaerobically through microbiological techniques but still half of them remain uncultivable [13]. Oral microbiome is characterized by cultivation-independent molecular methods using 16s RNA gene based cloning studies [13].

3. Oral microbiome variations in health and disease

The major gateway to human body is the oral cavity. Micro-organisms enter into the human cavity through food and air which passes through nose and then reaches trachea and lungs through mouth [3]. Oral microbiome causes number of oral infectious diseases such as dental caries, periodontitis, endodontic infection, alveolar bone loss and tonsillitis [3]. Studies have proved that an oral infectious disease affects the overall health of an individual, extending beyond the oral cavity such as systemic diseases including obstetric convolution [14], cardiovascular disease [15], immunological disorder [16], diabetes [17] and respiratory disease [18, 16]. Some of the major systemic implications are dealt with below.
3.1. Oral infection in Pre-term birth

Pregnancy gingivitis is a term to indicate inflammation which was promoted during pregnancy hormonal changes [19] or caused due to oral consumption of birth control pills [20]. Pre-term birth appears to be a risk factor for the neonatal mortality. *Bacteroides forsythus*, *Porphyromonas gingivalis*, *Agregatibacter actinomycetemcomitans*, *Treponema denticola*, *Fusobacterium nucleatum*, *Campylobacter rectus*, *Peptostreptococcus micros*, *Prevotella nigrescens* and *Prevotella intermedia* are the oral microbiome that are detected in high levels in the mothers of pre-term birth infants [21, 22]. The oral infection could mediate the pre-term birth and low birth weight in infants through one or more of the following mechanism such as, (1) Translocation of oral microbiomes to the feto-placental unit through inducing fetal or maternal response that result in the pre-term birth [23, 24]. (2) Systemic dissemination of prostaglandins and inflammatory mediators such as IL-6 (Interleukin - 6), IL-8 (Interleukin – 8), TNF – α (Tumor necrosis factor alpha) and PGE2 (Prostaglandin E2) on the fetal placental unit [24]. (3) Action of oral microbiomes reservoir of Lipopolysaccharides on the fetal placental unit [24].

Though there are sufficient evidences to prove that periodontal infection remains as a significant cause for the pre-term birth in infants it remains conflicting. However, oral hygiene during pregnancy is one of the preventive measures.

3.2. Oral infection and diabetes

Diabetes Mellitus (DM) is a clinical syndrome that is characterized by hyperglycemia which is caused due to deficiency of insulin. It affects all age groups. Type 2 diabetes mellitus substantially increases the risk of periodontal disease and has been proposed to modulate oral microbial communities. A shift to a more pathogenic bacterial profile may explain the greater risk of periodontal disease in diabetic patients. Animal studies provide support for such a shift as a mechanism for the increased risk of periodontitis in diabetes mellitus [25]. Studies have also shown that type 2 diabetes may alter subgingival bacterial community through changes in substrate availability driven by inflammation and glucose availability [26]. This could be driven by substrate-related alterations in the gingival sulcus in DM that may provide a microenvironment conducive to bacterial growth [27-30]. Consistent with this, a number of studies have shown that DM alters the colonization of various tissues by pathogenic bacteria. *Helicobacter pylori* infection [31], bacterial infection of foot ulcers, susceptibility to tuberculosis infection and colonization of the urinary tract by pathogenic bacteria are all significantly enhanced in diabetic individuals [32]. That DM enhances susceptibility to infection by pathogenic bacteria in many different target organs, this could be due to changes in bacterial substrate availability including sugars and inflammatory products that support bacterial growth. Though the link between oral infection and diabetes mellitus is not yet fully recognized and understood by medical community, there are many theories such as chronic hyperglycemia and increased secretion of prostaglandin E2 and Tumor necrosis factor alpha (TNF-α) are caused by the advanced glycation end products accumulation and presence of oral microbiome in the tissue due to impairment of polymorphonuclear leukocyte function [33, 34] and change in collagen metabolism due to increase in collagenase activity and decrease in collagen synthesis [35].

Many further studies are currently underway to determine the relationship between oral infection and diabetes in detail.

3.3. Oral infection and cardiovascular diseases

Cardiovascular disease (CVD) is a chronic disease that involves blood vessels or heart. Cardiovascular disease includes myocardial infarction, atherosclerosis, stroke and congestive heart failure due to genetic as well as environmental factors [36]. Apart from the above factors, oral microbiome infection also plays a significant role in triggering the cardiovascular disease. Among the periodontal bacteria, *Streptococcus sanguinis* and *Porphyromonas gingivalis* are commonly involved in cardiovascular disease [37].
The systemic inflammation of blood vessel wall in the presence of molecular mimicry and inflammatory mediator and inflammation by direct oral microbiome action such as direct invasion of bacteria to pocket wall and phagocyte-mediated bacterial translocation are the two modes by which the oral microbiome invades the vascular tissue forming acute inflammation, further forming resolution and homeostasis leading to chronic inflammation which causes cardiovascular disease [38].

4. Periodontitis and associated bacteria

Periodontal disease is one of the major ubiquitous diseases of the oral microbiome that causes tooth loss in adults [39]. The oral diseases are a disparate group of clinical entities where inflammation results in loss of attachment between the teeth and gingiva with the formation of periodontal pockets, which later leads to tooth loss contributing to tissue affliction [39, 40].

There are two main categories of periodontal disease where the tooth loses its endorsing structures are: aggressive periodontitis and chronic periodontitis. These diseases can be characterized beyond the extent of bone loss as generalized or localized and the austere of the disease as slight, moderate, or advanced [41]. Most of them suffer from chronic periodontitis, an insidious disease where the annihilation is consistent with the presence of bacterial plaque and mineralized plaque or calculus [42]. Chronic periodontitis is caused by the variable microbial patterns with polymicrobial infection. In deviance, aggressive periodontitis comprises of rapid attachment loss and bone destruction [43]. Localized form of aggressive periodontitis is an unusually unique disease relative to other forms of periodontitis which usually occurs during adolescence who usually shows a low incidence of periodontal disease; bone resorption in aggressive periodontitis progresses faster than that of observed in chronic periodontitis [44], may spontaneously arrest [45], and is localized to distinctive teeth (first molars and incisors); ensuing, the infection tends to aggregate together indicating susceptibility to the disease may be genetically regulated [46, 47].

Periodontal diseases commence with the aggregation of primary colonizers, usually facultative anaerobes and Gram-positive aerobes such as streptococci, onto the tooth surfaces. This colonization is succeeded by the additional aggregation of late colonizers, which are usually Gram-negative members of the “red complex”, namely T. denticola, T. forsythia, and P. gingivalis in addition to other Gram-negative organisms [48]. The broadly known periodontal pathogens present in plaque are Porphyromonas gingivalis, Treponema denticola, Prevotella intermedia, Campylobacter rectus, Tannerella forsythia, Aggregatibacter actinomycetemcomitans, Selenomonas spp., Fusobacterium nucleatum, Parvimonas micra and Eubacterium timidum [49]. Recent microbiome studies have disclosed emerging new pathogens as Filifactor alocis, which may play significant role in periodontal diseases [50]. There are increasing evidence to suggest that F. alocis plays an important role in community dynamics thereby could be a major player causing dysbiosis. The unique synergism between P. gingivalis and F. alocis were also noted in our earlier study [63].

5. Yet-un-cultivable bacteria and the recent shift in oral dysbiosis research

Recent studies conducted on oral microbiome over the last few years have altered our level of understanding the polymicrobial communities and their association to health and disease. Their increase in diversity of microbes and its composition gives an idea for identification and cultivation of more taxon than recognized previously. Some other bacterial species, such as Aggregatibacter actinomycetemcomitans, Prevotella intermedia, Selenomonas noxia, Eubacterium nodatum and Fusobacterium nucleatum are found to be correlated with periodontitis, along with the red-complex pathogens [51]. In addition, micro-organisms such as Desulfobulbus, Synergistes, Selenomonas, TM7 (new candidate bacterial division) and Filifactor alocis have been identified as potential pathogens [3]. Furthermore, 20% to 60% of the species identified in the oral cavity are yet to be cultivated [3, 52].

Recent theories on the etiology of periodontitis show a decrement in benign symbioses and an increase in micro-organisms with ameliorated pathogenic potential, favors a paradigm shift in microbial composition [53]. Hence, increment in oral microbiome diversity and its composition leads to conclude that pathogenic communities contain high levels of fastidious and yet-un-cultivated
bacteria than previously recognized [3]. Among them, Filifactor alocis [54, 55] is an emerging pathogen that is present in significantly high numbers in adult periodontitis, refractory periodontitis, and endodontic infections [56-58] and in aggressive periodontitis [59]. This bacterium has been identified as potential pathogen in a number of independent studies [3, 52, 60-62]. Aruni et al, [63] and Wang et al., [64] have described several of its potential virulence attributes, of F. alocis. Moreover, Chen et al., [65] recently reported that F. alocis was invariably present across various oral habitats in those with periodontitis. F. alocis both co-occurred with other pathogens and appeared to play a central role in organizing these pathogens.

5.1. Filifactor alocis

F. alocis is a Gram-positive, asaccharolytic, obligate anaerobic rod. Considered one of the marker organisms and an important periodontal pathogen. The organism is now identified to be significant to the pathogenic structure of biofilms associated with periodontal inflammation [43, 61, 62]. In comparison with the other traditional periodontal pathogens, the high incidence of F. alocis in the periodontal pocket compared with its absence in healthy individuals or those who are periodontitis-resistant has highlighted its importance in the infectious disease process [61, 62, 66]. This organism F. alocis, while heterogeneous, has virulence properties that may enhance its ability to survive and persist in the periodontal pocket [63]. Its relative resistance to oxidative stress and stimulated growth under those conditions are considered to be important attribute [63]. Furthermore, F. alocis has been shown to induce secretion of pro-inflammatory cytokines, triggering apoptosis of gingival epithelial cells [67]. Additionally, colonization and survival of F. alocis in a mouse model showed pro-apoptotic local infection that is rapidly resolved by host neutrophil influx [64]. Moreover, in co-culture with P. gingivalis, F. alocis showed an increased invasive capacity of HeLa cells [63]. Analysis of emerging research shows that F. alocis is a marker organism for periodontitis. It has unique characteristics that may enhance its virulence potential [18, 43, 53, 56, 63, 64, 67-71]. F. alocis could be one of the organisms that can play a pivotal role in community dynamics, establishing synergistic partnerships with other pathogenic oral bacteria during the disease state. In comparison with other Gram-positive bacteria of the oral cavity, the variations induced in the host proteome during F. alocis synergism could lead to many systemic host responses. Therefore, the significance of F. alocis putative virulence factors, which may trigger the key host response, deserves further intensive study. It is noteworthy that F. alocis is one of only a few organisms associated with both generalized and localized aggressive periodontitis (LAP) in addition to peri-implantitis and endodontic infections. Hence, F. alocis is considered an important species of the pathogenic oral microbiome.

6. Oral dysbiosis

Some diseases are caused due to change in the relationship of microbiome and the host; decrease in the beneficial symbionts wherein increasing the pathogenic potential causing microbiome imbalance inside the human body. This process is known as dysbiosis [72]. Oral dysbiosis is the gateway to periodontitis and its associated diseases.

7. Causes of Oral dysbiosis

Oral hygiene is the first and the foremost cause for dysbiosis of oral microbiome. Other major factors causing oral dysbiosis include poor oral hygiene, dietary habits, smoking, gingival inflammation, genetic difference and dysfunction of salivary glands such as activity of salivary proteins [73-75]. A dysbiotic shift and the microbiome imbalance in the oral cavity lead to formation of biofilm microbial community [76]. According to recent concept evident through earlier studies, it is known that in healthy host, the pathogens are found very few in numbers at the healthy sites and hence, the oral diseases are caused due to oral microbiome variations rather than exogenous infection [77]. Whereas in dysbiosis, the pathogenic bacteria grows remarkably high with anodyne components in biofilm surface [77]. This alteration in the formation of biofilm in the oral cavity results
in accumulation of large proportion of microbes as dental plaque biofilm [78]. Oral dysbiosis not only causes oral related diseases but also systemic diseases due to manipulation of host response.

The subgingival environment is rich in immune and inflammatory mediators and provides unique challenges and opportunities for the bacteria [79-81]. Periodontal health requires a controlled immuno-inflammatory state that can maintain host–microbe homeostasis in the periodontium [82]. However, in periodontitis, the host immune response is dysregulated either because it is subverted by the microbial community or because of host immunoregulatory defects and is therefore ineffective to restrain bacterial outgrowth and overt pathogenicity [11]. A poorly controlled host immune response, in turn, can generate a self-perpetuating pathogenic cycle where dysbiosis and inflammation reinforce each other. Among the immune cells, neutrophils represent the primary cellular defense in healthy oral tissues. They are the most common leukocytes recruited to the periodontal pocket and are indispensable for periodontal tissue homeostasis. Neutrophils are not adept at phagocytosing biofilm-associated bacteria, which eventually leads to ‘frustrated phagocytosis’ [83]. During this process, neutrophil-derived toxic substances may also be released to the underlying tissue, causing collateral damage to tooth-supportive tissues, as they function as double edged swords hence, a collateral damage can be exerted by hyperactive neutrophils or neutrophils in excessive numbers. Many bacteria especially P. gingivalis can subvert neutrophil functions and related immune responses causing dysbiotic community through impaired immune response. However, the role of its close synergistic bacterial counterparts (such as F. alocis) are yet to be studied in detail.

Specific molecular mechanisms by which periodontal bacteria manipulate the host response to cause dysbiotic inflammation are elaborately described in other reviews [83, 84].

Table 1. Table showing various mechanisms of immune subversion and their outcome [84].

<table>
<thead>
<tr>
<th>Mechanisms</th>
<th>Outcome</th>
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<tr>
<td>Whole cells, LPS bind to adhesion molecules (IL-8, ICAM-1, E-selectin).</td>
<td>Impaired recruitment</td>
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<tr>
<td>SerB suppression of IL-8 production by dephosphorylation of the Ser536 of</td>
<td>IL-8 production suppressed</td>
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<tr>
<td>NF-kB p65 preventing nuclear translocation and transcription.</td>
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<tr>
<td>Bacterial binding to FMLP and PPAD-citrullinated C5a.</td>
<td>Reduced chemotaxis</td>
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<tr>
<td>Dual regulation of TREM-1 by Arg- and Lys-gingipain. Outcome depends on</td>
<td>Evasion of host defense</td>
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<td>infection stage.</td>
<td></td>
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<tr>
<td>Resistance to killing by granular contents. C5 convertase-like activity</td>
<td>Killing prevented. Inhibits antimicrobial</td>
</tr>
<tr>
<td>produces C5a, which is involved in subversion of C5aR TLR2 crosstalk.</td>
<td>response and promotes inflammatory response</td>
</tr>
<tr>
<td>This leads to My88D degradation, PI3K activation and inhibition of RhoA</td>
<td></td>
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<tr>
<td>GTPase.</td>
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<tr>
<td>Activated CR3 interacts with P. gingivalis fimbriae and induces downregula-</td>
<td>Reduced bacterial clearance</td>
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<td>tion of IL-12p70 a key cytokine in intracellular bacterial clearance.</td>
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<tr>
<td>LPS and lipid A delay neutrophil apoptosis through TLR2 signalling</td>
<td>Prolonged acute inflammation</td>
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Recently studies from our lab has shown that the glycan modification due to oral bacterial sialoglycosidases (family of sialic acid modifying enzymes) could play a vital role in maturation of major virulence factors such as the gingipains and LPS. This in turn could play a role in sialic acid
8. Oral bacterial proteins involved in dysbiosis

Certain periodontal pathogens express major virulence proteins that impact apoptotic cell death in gingival epithelial cells (GEC) by disrupting cytokine networks. For example, it has been identified that major virulence proteins *F. alocis* and *P. gingivalis* modulate epithelial cells on co-infection and are responsible for host cell signaling, metabolic host response, cell-cell interaction, regulation of oncogenes in the oral microbiomes [18].

The up regulation of osteoclast pathway stimulation, increase in alveolar bone resorption and also tissue degradation through metalloproteinase and inflammatory mediators are induced through secretion of several cytokines by oral microbiome leading to disrupted cytokine homeostasis and finally tissue degradation [67]. Caspase-3 utilizing extrinsic pathway induce apoptosis and suppress MEK1/2 expression which may lead to apoptotic induction [85].

Apoptosis are triggered by secretion of certain proinflammatory cytokines, including tumor necrosis factor alpha (TNF-α), interleukin-1β (IL-1β) and IL-6 which are induced from gingival epithelial cells [67].

Several proteins namely, microbial surface component-recognizing adhesion matrix molecules (MSCRAMMs) contribute significantly in Gram-positive bacterial virulence by mediating precocious steps in clinical infection such as adhesion and colonization of host tissues [18]. Since host-pathogen interaction may support growth and modulation of metabolic processes the role of amino acid metabolism especially the arginine most prevalent in the periodontal niche may be responsible for the overall pathogenicity.

Proteome analysis plays a key role in understanding the process of dysbiotic inflammation through a better understanding of the related molecular mechanisms such as adhesion, invasion, pathogenesis, survival and adaptation in several oral pathogens such as *Streptococcus oralis* [86], *Streptococcus mutans* [87], *P. gingivalis* [88] and *Fusobacterium nucleatum* [89].

Proteome analysis of *F. alocis* showed several proteases such as CaaX proteases, metal dependent proteases, calcium dependent proteases and sialoglycoproteases (http://www.ncbi.nlm.nih.gov/genomeprj/46625). Expression of these proteases was found to be elevated in co-culture with *P. gingivalis*. Proteins such as Acetyl glutamate kinase, Ornithine transaminase, Aminotransferase and Glutamate racemase were identified to be involved in ornithine biosynthesis. Several proteins contributing to virulence potential of *F. alocis* were identified such as leucotoxin translocation ATP-binding protein, CBARP (Voltage dependent calcium channel beta subunit-associated regulatory protein), fibronectin-binding protein, fimbrial assembly protein, Type IV pilus assembly protein, toxin–antitoxin component protein, Hemolysin III type calcium-binding protein and NAPA (Neutrophil Activating Protein A) [68].

*F. alocis* proteome contain proteins involved in ornithine biosynthesis and catabolism, urea breakdown such as arginine deiminase, ornithine transaminase, acetyl glutamate kinase, glutamate racemase, amidotransferase, arginine-tRNA ligase, aminotransferase, arginine decarboxylase [68]. *F. alocis* OTC (Ornithine transcarbamylase) has also shown to be involved in the citrullination of proteins via the ADI (Arginine Deiminase) pathway. Since protein catabolism is an alternative source of energy in asaccharolytic oral bacteria, heavy amounts of ammonia production occur in periodontal pockets leading to oral dysbiosis [68].

Novel proteins of *P. gingivalis* namely the sialoglycosidases that interact with sialic acid of the host cells have been recently considered to play an important role in facilitating dysbiosis [90]. In the saliva rich environment the mucus containing glycans are considered a vital source as energy for bacterial growth and survival. Manipulation of the sialic acid is recently considered a mechanism to trigger immune evasion through prevention of sialic acid – siglec (Sialic acid binding lectins) interactions in neutrophils.

Major proteins involved in citrullination have been identified to cause post translational modifications in the host predisposing to rheumatoid arthritis. One of our study has identified...
arginine deaminase of F. alocis to be involved in a process similar to the peptidyl arginine deiminase of P. gingivalis causing peptidyl citrullination [69].

9. Proteins of oral bacteria related to systemic diseases

Apart from the proteins of oral microbiome that causes oral related disease, there are several other oral bacteria proteins that cause systemic diseases. Proinflammatory mediators such as IL-1β (Interleukin-1β), TNF-α (Tumor necrosis factor – α) and PGE2 (Prostaglandin E2) are released in high levels that exaggerate the host response to the microbes that causes systemic infection in the humans [91, 92]. In case of preterm birth, an elevated levels of cytokine IL-6 has been identified in the host along with IL-1β, TNF-α and PGE2, that causes the infection in the amniotic fluid which leads to complication in the delivery [93].

Studies shows that, a protease namely Gingipain R released from P.gingivalis in large quantities causes cardiovascular disease by activating factor X, prothrombin and protein C, thus thrombotic tendency is promoted as thrombin is released, aggregation of subsequent platelets, transformation of fibrinogen to fibrin and intravascular clot formation [21]. Also, recent studies indicate that chronic oral infection induces high proportion of Hsp65 (Heat Shock Protein) that increases cardiovascular risks [94, 95].

Many oral microbiome proteins related to systemic diseases still remain as hypothetical proteins.

Their functions need to be determined.

10. Stem cell modulation by oral pathobionts

One of the major effects of pathogenic oral microbiome is manifested in the oral stem cell modulatory processes. Our preliminary study showed secretary proteins of P. gingivalis modulated stem cell characteristics. Stem cells contribute to host defense and inflammation. During bacterial - inflammatory disease, stem cells are subjected towards the site of damage, hence come close to bacteria and its components. Previous studies using epithelial cells infected by bacteria have showed modulation of various important functional pathways and changes in gene regulation [63]. Extracellular bacterial proteins secreted in a chronic bacterial infection could modulate stem cells and affect tissue regeneration. Our studies using transcriptomic approach employing induced pluripotent stem cell iPSCs (Induced Pluripotent Stem Cells) were used to evaluate the phenotypic and molecular characteristics. The iPSCs were incubated in different experimental conditions with the secreted proteins of pathogenic bacteria - Porphyromonas gingivalis, a commensal bacterium - Enterococcus faecalis and a beneficial bacterium - Lactobacillus casei to comparatively evaluate the expression of key genes that govern stemness and differentiation. Secretory proteins of pathogenic bacteria - P. gingivalis, were found to possess a modulatory effect both on the stemness and the differentiation of stem cells. It is likely that during a chronic bacterial infection, this could prolong stemness and prevent differentiation. Hence, sustain infectious state and prevent cell recovery.

11. Animal models for studying systemic diseases caused due to periodontitis

Experimentally induced animal models are critically important which is used to study and analysis different aspects of the oral disease and also the systemic diseases caused due to oral dysbiosis. Though human cell culture or in-vitro cell culture were found to be useful models, the findings about the host-microbe interaction was anonymous and obscure and hence the animal model (in-vivo) came into limeligh [96]. A detailed review on this topic can be found in [96].

Nonhuman Primates have similar oral structures as that of humans. In particular, rhesus monkey (Macaca mulatta), cynomolgus monkeys (Macaca fascicularis) and baboons (Papioanubis) are susceptible to disease. They are inoculated with human pathogens and then tested. But, they are prone to tuberculosis which makes them less practical model [97].

Minnesota Miniature Pigs have maxillofacial and oral structures analogous to those of humans in terms of physiology, anatomy and progression of disease [98]. However, miniatures pigs are relatively expensive and only very few studies are available to support their use [96].
The host-microbiome interactions in the rodent model are similar to the human. Rats are often used as an effective model. They are inexpensive and obtained easily with different microbial status. Sometimes swamp rice rats (*Oryzomys palustris*) are widely used to assess some therapeutic models and the dietary effects [96, 98].

Baker mouse model, chemically induced model and murine back abscess model are used to examine the alveolar bone resorption, inflammatory response and the interaction of host response and oral microbiome to various phylotypes that leads to systemic disease [99] respectively. But, since they are small in size, they are needed in large quantities.

Rabbits, Ferrets and Hamsters are also used as animal models. Each of the animal models has its own advantage and disadvantage, though most of them show similarities to human disease. Among these, mice and rats models are useful for understanding certain aspects of host-microbiome interaction and their therapies. They are being successfully used to study the “dysiosis-rebiosis” concept of oral health restoration.

Though the animal models provide a huge number of findings and data, it is occasionally arduous to ascertain whether the findings and data are pertinent to human [96].

**12. Conclusion**

Recent studies pave a paradigm shift in emphasizing oral ecosystem to be vital to maintaining oral and overall health of the body. Maintaining microbial equilibrium within oral cavity protect pathogens from manifesting disease state. Disturbing homeostasis of the oral cavity can flare pathogen activity leading to disease state. Because oral cavity being the primary gateway to body may result in spreading infection to other body sites producing systemic diseases. What is known until now is all about oral bacterial species that can be cultivated and sequenced and that can be obtained from pure culture approaches. However, this does not reflect their actual behavior in complex microbial communities. Because, the species that have been identified by culture independent methods are still classified as uncultivated phylotypes. Their impact on community dynamics and their role in oral dysbiosis is yet to be fully explored. Furthermore, newer concepts to study dysbiosis through immune subversion mechanisms involving gycan mediated pathogen-host interactions is currently being explored. Recent research focus on bacteria such as *F. alocis* and other new candidates are gaining momentum. Their collective role in community dynamics is yet to be clearly explored. Hence, an insight into community level physiological and metabolic capacity will help find new cure to modulate activity of such communities and steer them forwards health state. Their synergistic and competitive contributions to oral dysbiosis could reveal new insight into specific species, or its genes or pathways of interest to develop novel therapeutics. Analysis of human oral microbiome will significantly contribute to the development of precise medical interventions.

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**Author Contributions:** PS - worked on the major contribution, typing, correction and revisions of the manuscript, AG,AB- section contribution, AW- overall manuscript revision.

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