REVIEW ARTICLE

CONTENTS

# Microbiology of dental decay and periodontal disease: A review

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## ABSTRACT

**Aim:** This review attempts to examine the microbiology, pathogenesis and current therapeutic approaches of dental caries and periodontal diseases with a special focus on the role of polymicrobial biofilms, the host-microbe interaction and the major pathogenic species involved in disease progression.

**Materials and Methods:** A thorough literature review was performed using major scientific databases such as PubMed, Scopus, Web of Science and Google Scholar. Studies that were published between 2000 and 2025 were included. Relevant experimental, clinical and review articles that focused on the etiology, microbial composition, virulence mechanisms, host immune responses and therapeutic approaches of dental caries and periodontal disease were analyzed.

**Conclusions:** The oral cavity harbors over 700-800 bacterial species, of which the primary cariogenic pathogen is *Streptococcus* mutans and *Porphyromonas* gingivalis has been implicated as a major cause of periodontal disease. Dental caries progression is mostly attributed to acid production and demineralization of enamel, whereas periodontal disease is a result of dysbiotic shift in the subgingival microbiome with destructive host inflammatory responses. The "red complex" (*P. gingivalis, Treponema denticola and Tannerella forsythia*) has a high degree of synergistic virulence in advanced periodontitis. Biofilm formation, production of extracellular polysaccharide (EPS) matrix, quorum sensing and immune components (neutrophils, macrophages and matrix metalloproteinases or MMPs) are all factors that contribute to disease formation. Prevention strategies include oral hygiene measures, fluoride exposure, dietary modification, and antimicrobial agents, whereas treatment measures include mechanical debridement, systemic antibiotics, antimicrobial peptides, probiotics, and photodynamic therapy. Dental caries and periodontal diseases are the result of complex interactions between polymicrobial biofilms and immune responses by the host. A better understanding of the microbial ecology, virulence pathways and host-pathogen interactions is crucial in the process of improving prevention and treatment. Advances in targeted antimicrobial therapies and innovative therapeutic approaches hold promise for enhancing global oral health outcomes.

**KEY WORDS:** dental carriers, periodontal disease, biofilm, *Streptococcus mutant*, quorum sensing

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## INTRODUCTION

Periodontal diseases and dental caries are the most common chronic infectious complications of the mouth cavity in the world, which place significant burdens on the health system of an individual and the population at large. Both pathologies are due to complicated biofilm-mediated mechanisms whereby the resident microbiome of the oral cavity alters its state of being symbiotic and homeostatic to a state of dysbiosis and pathogenicity. The microbial ecology, molecular mechanisms and host-microbe interactions that contribute to these diseases are important to understand so that evidence-based preventive and therapeutic interventions can be developed [2].

The oral microbiota can be defined as a dynamic and diverse ecosystem of microbes consisting of about 500 to 700 different bacterial species, which further contributes to fungal and viral species to oral health or disease states. Unlike the historical conception of oral disease as resulting from single pathogenic agents, modern microbiological knowledge of oral diseases is based on the now commonly accepted view that dental caries and periodontitis are polymicrobial diseases that involve complex synergistic interactions involving multiple microorganisms, with individual host factors and environmental factors determining the clinical manifestation and progression of these diseases [1].

Dental caries has come to be recognised as a biofilm-mediated, sugar driven, multifactorial disease involving the progressive demineralisation of dentine and enamel caused by persistent acid production from acidogenic and aciduric microbial communities. Rather than being caused by a single pathogen, caries results from ecological changes that take place in supragingival dental plaque biofilm communities in which high frequency consumption of fermentable carbohydrates (especially sucrose, refined sugars) selects for acid producers such as *Streptococcus mutans*, *lactobacilli,*

*Actinomyces, Bifidobacterium, and Veillonella,* while inhibiting acid-sensitive commensal organisms. These cariogenic communities have three cardinal virulence traits: acidogenicity (rapid fermentation of dietary carbohydrates into organic acids), aciduricity (survival and metabolic activity in low pH) and robust extracellular polysaccharide (EPS) synthesis that increases biofilm adhesion, structural integrity and diffusion resistance [3].

In contrast, periodontal diseases (including gingivitis and periodontitis) have been linked to dysbiotic subgingival biofilms enriched in Gram-negative anaerobes and proteolytic species that are responsible for chronic inflammation, irreversible destruction of the connective tissue and resorption of alveolar bone. Pathogenesis Periodontitis is not only caused by the growth of the total bacterial biomass, but also by changes in microbial composition and activities, which are frequently mediated by low-abundance keystone pathogens like Porphyromonas gingivalis.

The pathogenesis of periodontitis is driven not merely by an increase in total bacterial biomass but by qualitative shifts in microbial composition and function, often orchestrated by low-abundance "keystone pathogens" such as *Porphyromonas gingivalis*. These organisms manipulate host immune signaling, specifically the complement signaling and TLR signaling, to induce an inflammatory environment that favors the outgrowth of proteolytic and asaccharolytic bacteria as well as nutrient-rich conditions through gingival crevicular fluid and perpetuates tissue breakdown through bacterial proteases and gingipains as well as host matrix metalloproteases.

The progression from health to disease, both in caries and periodontitis can be explained by the ecological plaque hypothesis, which holds that environmental stressors such as frequent sugar consumption, low salivary flow, poor oral hygiene, smoking, diabetes, and immunocompromise affect the state of microbial homeostasis and select for pathogenic communities. Advances in high-throughput sequencing, metagenomics, metatranscriptomics, and metabolomics have shown that both diseases are polymicrobial and community-level diseases with unique dysbiotic signatures in terms of altered species diversity, functional gene expression, and metabolic products. Notably, oral dysbiosis has a more extensive pathophysiology systemic effect because pathogen-associated oral biofilms are reservoirs of respiratory pathogens, inflammatory cytokines and proteolytic enzymes that cause cardiovascular disease, diabetes, rheumatoid arthritis, adverse pregnancy outcomes, and respiratory infections [4].

The present review gives a concise overview of the available microbiological knowledge of dental caries and periodontal disease, its structure, pathogenicity, biofilm structure and dynamics, bacterial communication systems, host immune response, and the recent prevention and treatment options. Microbiological, immunological, and clinical perspectives integration offers a basis for interpreting available literature and designating future research objectives and treatment possibilities in oral disease management.

## AIM

The purpose of the review is to assess the microbiology, pathogenesis, and current treatment regimens of dental caries and periodontal disease, along with the role of multispecies biofilms, host-microbe interactions, and pathogenic microorganisms that play a role in the progression of the disease. The study is also aimed at giving in depth insight into the correlation between oral microbes and host immunity to guide the progress in developing improved preventive and therapeutic interventions.

## MATERIALS AND METHODS

Major databases were used to conduct a systematic literature review, i.e., PubMed, Scopus, Web of Science, and Google Scholar.

Studies on the period 2000-2025 were included and were experimental, clinical and review articles addressing:

- Etiological agents of dental caries and periodontal diseases,
- Composition and dynamics of oral microbiota,
- Mechanisms of biofilm formation and interspecies interactions,
- Host immune responses,
- Preventive and therapeutic strategies.

Relevant studies were critically reviewed in order to present a comprehensive overview of the most recent scientific findings on oral microbiology and its association with dental caries and periodontal diseases.

## REVIEW AND DISCUSSION

### OVERVIEW OF ORAL MICROBIOTA

#### *COMPOSITION AND DIVERSITY*

The human oral cavity is one of the most complex and dynamic microbial ecosystems that is associated with human health. The oral microbiota includes bacteria, archaea, fungi, viruses and bacteriophages, and bacteria are a dominant component of the oral microbiota.

The monumental diversity of the oral microorganisms has been demonstrated by cultivation-independent molecular methods, especially 16S ribosomal RNA gene sequencing and metagenomics. The oral microbiota contains over 700-800 species of seven major phyla, such as *Actinomycetota (formerly Actinobacteria), Bacteroidota (Bacteroidetes), Bacillota (Firmicutes), Fusobacteriota, Pseudomonadota (Proteobacteria), Saccharibacteria* and *Spirochaetota*. Recent metagenomic catalogs have revealed more than 3,400 species-level clusters containing about 60 percent of uncharacterized taxa, suggesting that most of the oral microbial diversity is uncultivated and poorly characterized [5].

In addition to bacteria, the oral microbiome harbors various non-bacterial microorganisms, which play an ecological role and pathogenesis of diseases. Fungi, predominantly *Candida* species, are detected in ~70% of healthy individuals and can form biofilms, with over 150 species documented in the oral mycobiome. The archaea, mainly methanogens e.g. *Methanobrevibacter* oralis, are found in low levels in health, but in periodontal disease and in peri-implantitis, they may work synergistically with the pathogens. The most common viral element is bacteriophages, more than 60,000 phage groups by the species are known; most of these are temperate phages that actively control the growth of bacteria and mediate horizontal gene transfer and genetic variation. The functions of these non-bacterial elements in oral disease and health are not fully studied yet [6].

The oral microbiota is very site-specific, and dental plaque and tongue dorsum, buccal mucosa, saliva and gingival sulcus communities exhibit different oxygen tension, nutrient availability, pH, and salivary flow. The most diversity is found in supragingival and subgingival plaques, with subgingival plaque having less oxygen and more anaerobiosis. Fluorescence in situ hybridization (FISH) biogeographic studies have shown that oral biofilms are organized at micron scales and certain genera of bacteria have a distinct location and multispecies consortia [7].

Primary colonizers of the oral cavity are bacteria initially colonizers of the oral cavity, which are mostly Gram-positive bacteria, mainly streptococci: *Streptococcus sanguinis*, *Streptococcus oralis*, *Streptococcus mitis* (the mitis group), and the species of *Actinomyces*, *Haemophilus*, and *Neisseria*. These organisms can stick to tooth surfaces that are covered by salivary pellicles and form early biofilms.(29). Primary colonizers also generate extracellular enzymes and metabolic products that alter the local microenvironment, decreasing oxygen tension and pH, and allowing secondary colonization by the fastidious anaerobes such as the members of the red and orange bacterial complexes within a few hours of biofilm formation [8].

There is significant compositional complexity of the subgingival microbiota, and anaerobic species are predominant because of the oxygen-deprived nature of the periodontal pocket. The important subgingival microbes are the members of the red complex *(Porphyromonas gingivalis, Treponema denticola, Tannerella forsythia)*, the orange complex flora (*Fusobacterium nucleatum*, *Prevotella intermedia*, *Prevotella nigrescens*, *Peptostreptococcus micros*), and the yellow complex organisms (many species of *Streptococcus* such as *S. mitis*, *S. oralis*, *S. sanguinis*, *S. gordonii*, *S. intermedius*) [4, 9].

The oral biofilm is spatially organized by having specific bacterial aggregates and layered structures spread throughout the matrix to provide micro environments with different levels of PH and oxygen tension, and varying nutrient levels.

The stratification of subgingival plaque has been shown to be divided into various morphologic layers, the most important layer of which is the basal layer that is close to the tooth surface, the intermediate layer with a high number of filamentous bacteria, Corynebacterium and Actinomyces, and the outermost layer composed of a high percentage of motile spirochetes and other late colonizers. This spatial arrangement records metabolic collaboration and substrate trade among the species and is essential in the functions of a biofilm as well as pathogenicity [10]

## *ORAL MICROBIOTA IN HEALTH VERSUS DISEASE*

In periodontally healthy conditions, the oral microbiota is symbiotic in contact with host tissues, in complex metabolic interrelationship and immune tolerance mechanisms. Healthy oral biofilms have a rich microbial diversity, resident commensals excluding colonization by pathogenic species by competitive exclusion, antimicrobial compound production, and creation of local environmental conditions adverse to pathogenic species [2].

The pathological change in the composition of microbial communities, dysbiosis, is an indicator of a shift between an oral health state and a disease state. The microbial diversity in dysbiotic biofilms related to caries is reduced, whereas acid-tolerant and acidogenic bacteria such as *Streptococcus mutans*, *Lactobacillus species*, and *acid-tolerant streptococci* increase in number. These organisms cause acidic micro environments favorable to their existence but unfavorable to acid sensitive species, triggering a self-reinforcing loop of dysbiosis [1].

Shifts in microbial composition towards pathogenic consortia that are enriched in proteolytic species and

can invade tissues and synthesize virulence factors are present in periodontal disease as dysbiosis. It is not merely an increase in the abundance of pathogens that is involved in health-disease transitions, but rather a fundamental restructuring of microbial community structure, changes in the pattern of gene expression and a shift in metabolic interactions among biofilm constituents [2].

## DENTAL CARIES: MICROBIOLOGY AND PATHOGENESIS

### *ETIOLOGY AND DISEASE MECHANISM*

Dental caries is a biofilm-mediated pathology that develops as a consequence of the interaction between cariogenic microbes, dietary carbohydrates, especially sucrose and host factors. There is a series of steps in the disease process that involve the colonization of the tooth surfaces by bacteria, formation of biofilms, fermentation of carbohydrates to produce organic acids and demineralization of the tooth structures. The pathogenesis of caries is the classical three-factor triad, including susceptible host (saliva composition or flow), cariogenic bacteria with an ability to convert carbon dioxide into acids and withstand acid levels), and the frequent intake of carbohydrates to feed bacteria [1, 11].

Special attention should be paid to the role of the dietary sucrose. The ability of sucrose to be the preferred substrate for extracellular polysaccharide (EPS) synthesis is the reason behind its special status as compared with other fermentable carbohydrates. Although all fermentable carbohydrates produce acids due to the activity of the bacterial metabolism, the characteristic structural features of sucrose allow enzymes of glucosyltransferase (Gtfs) of *Streptococcus mutans* to break down sucrose to glucose and fructose units and produce glucose polymers (glucans) in a particular 1,3 and 1,6 glycosidic structure. S. *mutans* express three different Gtfs: GtfB expresses mostly water-insoluble, 1,3-linked glucans, which enable strong adhesion of the bacteria to the surface and give the biofilm structure a rigidity; GtfC expresses a mix of soluble and insoluble glucans; GtfD expresses primarily soluble 1,6-linked glucans, which are used as extracellular energy stores [12].

### *PRINCIPAL PATHOGENIC ORGANISMS*

*Streptococcus* mutans holds a superior role as the main etiological cause of dental caries. This is a Gram-positive, facultative anaerobic coccus that has several cariogenic pathogenicity features. This is because *Streptococcus* mutans synthesizes glucosyltransferase (Gtf) enzymes that mediate the production of insoluble and soluble glucans on sucrose to produce an extraordinarily adhesive biofilm matrix. The organism is truly acid-tolerant with a viability and even growth down to pHs of 3.5, which is nearly unique in oral bacteria[1, 13].

*Streptococcus* mutans is present as serotype c, e, f, and k of which serotype c is the most common oral serotype with about 70-80% of oral S. mutans isolates having this serotype whereas serotype e has about 20, serotype f and k each form represents less than 5% cases [1]. These serotypes differ in their virulence properties, geographic distribution and other oral isolates in their association with extraoral pathologies; some serotype k and some serotype e strains are found more abundantly in cardiovascular specimens and cases of infective endocarditis than oral isolates, indicating a potentially higher invasive capacity. A small proportion of strains are unable to be typed using conventional PCR methods, which reflects a lack of RGP diversity beyond the standard four serotypes; however, some clinical isolates are dual serotype (commonly c and k) and thus display diverse RGP populations [14].

Other secondary pathogenic agents that help in the pathogenesis of caries are *Streptococcus sobrinus*, which is also a mutans streptococci member and a number of species of *Lactobacillus*. The role of the latter organisms on caries pathogenesis is yet to be fully defined [15].

### *VIRULENCE FACTORS AND PATHOGENIC MECHANISMS*

*Streptococcus* mutans has numerous virulence factors coordinating its cariogenic capability. The glucosyltransferase enzyme family is an important virulence factor. The glucan synthesis involved three main Gtf enzymes (GtfB, GtfC, and GtfD), which have different linkage specificities: GtfB synthesizes insoluble α1,3-linked glucans, which shape the biofilm matrix, GtfC synthesizes soluble and insoluble α1,6-linked glucans [12]. The glucans containing heterogeneous biofilm microarchitecture enabling colonization by microbes and structural stability is determined by the compartmentalized distribution of glucans with dissimilar linkage features [16].

The synthesis of fructans by the use of fructosyltransferase (Ftf) enzymes catalyzes the production of further matrix polysaccharides and is also an ancillary virulence mechanism that uses fructose as its substrate. GbpA, GbpB and GbpC are glucan-binding proteins (Gbp) that facilitate bacterial adherence to glucan molecules on the surface of the bacterium, connecting microbial cells with the scaffold of the matrix. The regulation of these

virulence determinants via a gene is through a series of regulatory systems, such as the AtpF (F1F0-ATPase) and LuxS (Autoinducer-2 production) and ComABCDE systems (competence and biofilm regulation), which coordinate the expression of virulence factors in response to environmental signals [1].

*Carbohydrate* fermentation resulting in acid production is one of the key pathogenic processes. The dentin is directly eroded by lactic acid and other organic acids produced by Streptococcus mutans by glycolytic pathways at acidic pH. The organism stores intracellular polysaccharides, which are formed using various carbohydrates, this allows it to produce acid even when carbohydrates are starved. This intracellular store of polysaccharides maintains the ability of the organism to produce acid over a long period, continuing to maintain the acidic microenvironment despite the scanty external supply of carbohydrates [1, 13].

## PERIODONTAL DISEASE: MICROBIOLOGY AND PATHOGENESIS

### *DISEASE CLASSIFICATION AND PROGRESSION*

Periodontal diseases represent a continuum of inflammatory diseases of tissues of the tooth support, which include gingivitis (inflammation of gingiva tissue without loss of underlying alveolar bone) and periodontitis (loss of alveolar bone and periodontal attachment). Periodontitis is divided into aggressive periodontitis (faster developmental stages, the age of the patient at the time of the onset of the disease) and chronic periodontitis (slower development, mostly in middle-aged and elderly people). Periodontal disease pathogenesis entails a polymicrobial threat to periodontal tissue and an untamed host inflammatory reaction that leads to the deterioration of periodontal tissues [2, 17].

### *THE RED COMPLEX AND ORANGE COMPLEX BACTERIAL CONSORTIA*

Modern periodontal microbiology has conceptualized the pathogenic bacteria as being formed into color-coded groups of bacteria on the basis of association with each other, and temporal emergence through biofilm formation was introduced by Socransky et al in 1998. The orange complex has such secondary colonizers as *Fusobacterium nucleatum, Prevotella intermedia, Prevotella nigrescens, Peptostreptococcus micros, Eubacterium nodatum, Campylobacter rectus, and Streptococcus constellatus.* They mediate the transition between early colonizing streptococci and actinomyces and late colonizing red complex pathogens, and are scaffolding bacteria that form microenvironmental conditions (deoxygenated, increased nutrients of gingivally secreted crevicular fluid) that are conducive to the highly fastidious anaerobes [8]. There are three pathogenic species forming the red complex: *Porphyromonas gingivalis, Treponema denticola and Tannerella forsythia (formerly Bacteroides forsythus*). They are the most significant pathogens of adult periodontal disease and have a strong relationship with the clinical development of advanced periodontitis [18]. The red complex bacteria do not occur in isolation; instead, they are usually present in periodontal pockets in synergistic polymicrobial consortia. Complex anaerobic fermentation of amino acids, extracellular proteolytic activity, the synthesis of toxic metabolites such as lipopolysaccharides and outer membrane vesicles, and increased interspecies virulence are the homologous pathogenic features [19].

### *PORPHYROMONAS GINGIVALIS: THE KEYSTONE PATHOGEN*

*Porphyromonas* gingivalis has come to be a model of a key pathogen in periodontitis- an organism that is found in comparatively low abundance but has a disproportionate effect on the biofilm microbial composition and virulence by a variety of immune subversion mechanisms. This is an obligate asaccharolytic, anaerobic, Gram-negative rod that expresses a wide range of virulence factors that allow it to penetrate tissues, avoid the immune response, and promote pathobiont status [2].

Gingipains (Rgp and Kgp arginine-specific and lysine-specific cysteine proteases) are some of the virulence factors in *porphyromonas* gingivalis, which are some of the important pathogenic determinants. Gingipains destroy host proteins such as immunoglobulins, complement, cytokines, and chemokines that inhibit host defence systems and produce peptides and amino acids to aid bacterial metabolism. This organism synthesizes lipopolysaccharides that have distinctive lipid A frameworks that cause attenuated Toll-like receptor 4 signaling, which controls host inflammatory reactions [20]. Major fimbriae (FimA) mediate epithelial cell invasion and co-aggregation with other bacterial species, whereas minor fimbriae (mfa) mediate biofilm formation [19].

Particularly notable is *P. gingivalis'* inflammophilic nature—the organism thrives within inflammatory environments, utilizing hemorrhage products and inflammatory exudates as nutrient sources. The production of complement and cytokines is mediated by the bacterial lipopolysaccharide and peptidoglycan that produce nutritionally advantaged inflammatory microenvironments. Additionally, *P. gingivalis* actively evades antimicrobial

responses such as neutrophil oxidative burst and phagocytosis by a variety of mechanisms, such as degradation of the TLR2 adaptor protein MyD88 by cysteine proteases, which facilitates disease-sustaining dysbiosis [2].

### *TREPONEMA DENTICOLA AND TANNERELLA FORSYTHIA*

*Treponema denticola* is a motile spirochete which also leads to the pathogenicity of the red complex in several ways. The organism secretes hydrolytic enzymes such as collagenases, hyaluronidases and proteins that directly break down host extra cellular matrix proteins. It is also important to note that *T. denticola* shows nutritional interdependence with *P. gingivalis*: the latter organism synthesizes isobutyric acid, which favors the growth of *T. denticola*, and *T. denticola* produces succinic acid, which favors the growth of *P. gingivalis*. This example of metabolic cooperation is an example of polymicrobial synergism in periodontal biofilms [2].

*Tannerella forsythia* is an organism that has been recovered as a late colonizer in progressive periodontal disease and also produces dentilisin (a serine protease), collagenase and other proteolytic enzymes involved in degrading periodontal tissue. It is covered with special O-glycans, which mediate the immune evasion and survival of the organism in periodontal pockets. Interestingly, *T. forsythia* is often colonized prior to *P. gingivalis* colonization in subgingival biofilms, indicating primary ecological colonization functions [18].

## BIOFILM FORMATION AND MATRIX COMPOSITION

### *STAGES OF BIOFILM DEVELOPMENT*

The formation of dental plaque biofilm occurs in a series of sequential phases with each stage defined by a particular microbial community, composition of the matrix and its functional features. Knowledge of these developmental stages can give information on intervention points in biofilm control and disease prevention [21].

*Stage 1: Pellicle formation and initial microbial attachment*
When teeth are cleaned or ejected to the oral cavity the acquired salivary pellicle is quickly colonized by the salivary pellicle, a proteinaceous film of more than 180 proteins, glycoproteins, phosphoproteins and lipids. Pellicle formation occurs several seconds after exposure of the tooth surface to saliva, with precursor proteins being proline-rich proteins, statherin, and histatins binding directly to the hydroxyapatite crystals via calcium-mediated interactions. The formation of pellicle begins with a pellicle thickness of roughly 10-20 nm within minutes, the growth of the pellicle through protein-protein interactions occurs after 30-45 minutes, and the maturation of the pellicle occurs after 90-120 minutes as the adsorption of high-molecular-weight mucins onto the pellicle structure occurs [22]. This is due to the fact that pellicle composition has a very strong effect on the later colonization by microbes. Salivary pellicle gives certain receptors mediating bacterial adherence: glycosylated proteins and histatins mediate streptococcal adhesion, proline-rich proteins mediate Actinomyces species adhesion, whereas statherin mediates Fusobacterium nucleatum adhesion. Enzymes that are functionally active, such as peroxidases, lysozyme, and amylase are also found in the pellicle and regulate adhering bacterial physiology [22].

*Stage 2: Primary colonization and microcolony formation*
Gram-positive facultative bacteria, especially streptococci and actinomyces are the ones that preferentially colonize pellicle-coated surfaces in 2-4 hours of pellicle formation. These major colonizers divide further to create microcolonies of the growing biofilm. Primary colonizers show glycosidase activities, which allows them to get access to the salivary glycoproteins as a nutritional source. Preventing the elimination of other anaerobic species through the initial metabolic operations of oxygen-depleted microenvironment is enabled by the process of oxygen consumption. Primary colonizers produce novel bacterial cell surface receptors that permit secondary bacterial adhesion by coaggregation, where bacteria, by complementary adhesin-receptor interactions [21].

*Stage 3: Secondary colonization and biofilm maturation*
Primary colonizers are bound by secondary colonizers which include Fusobacterium nucleatum and other anaerobic species via coaggregation. Fusobacterium nucleatum is a so-called coaggregation hub that interacts with nearly all oral bacterial species, thus facilitating multispecies biofilm formation. Subsequent growth of biofilm provides heterogeneous micro environments that have oxygen tension, pH, nutrient, and metabolic concentration gradients. The maturation of biofilms is characterized by the formation of 3-dimensional design with mushroom or clumped microcolonies embedded in extra-cellular matrix [22].

### *EXTRACELLULAR POLYMERIC SUBSTANCE MATRIX COMPOSITION*

Extracellular polymeric substances (EPS) that include polysaccharides, proteins, extracellular DNA (eDNA), and lipids predominate, but cell wall polymers, including lipoteichoic acids and peptidoglycan fragments are present

in lesser quantities in the biofilm extracellular matrix. EPS is particularly highly concentrated with insoluble and soluble glucans and fructans produced by Streptococcus mutans glucosyltransferases (GtfB, GtfC, GtfD) and fructosyltransferase (Ftf) in cariogenic supra-gingival plaque, creating a diffusion restrictive, mechanically stiff matrix to facilitate local bacterial concentration and acid retention. By comparison, the periodontitis-associated biofilms subgingival matrix has a more complex biofilm composition (bacterial and host-derived components: polysaccharides, proteins, eDNA, lipids, serum proteins, and inflammatory exudate), although its actual chemical composition is not so well defined [23].

## *POLYSACCHARIDE COMPONENTS*

Exopolysaccharides are large matrix constituents that are found as glucans and fructans in cariogenic biofilms. The glucosyltransferase enzymes of *Streptococcus mutans* produce glucans of various structures: α1,3-linked insoluble glucans are the major scaffold of the matrix, and α1, 6-linked soluble glucans are the secondary binding sites. The glucan matrix has a compartmentalized structure which has spatial heterogeneity that forms different microenvironments. Fructose is further enriched with fructan constituents produced by fructosyltransferase [11, 12].

Exopolysaccharide compositions also vary significantly in subgingival biofilms related to periodontitis compared to cariogenic biofilm maturation, but the present knowledge about periodontal biofilm matrix polysaccharides is incomplete. But, there are bacterial species such as *Prevotella* and *Fusobacterium* species that produce exopolysaccharides, which lead to the formation of the matrix.

## *PROTEIN COMPONENTS*

Proteins are 10-20% of the biofilm mass that is of bacterial and host origins. The bacterial surface protein such as glucan-binding proteins, adhesins (especially antigen I/II and other cell-surface proteins) and enzymes are incorporated into the matrix. Amyloid structures are produced by a large number of surface proteins, which may have a structural role in assembling the matrix. Host-derived proteins such as immunoglobulins, complement components and tissue-derived proteins are deposited in biofilm matrices either by entrapment or active integration [23].

## *EXTRACELLULAR DNA*

Extracellular DNA (eDNA) makes up 1- 20 percent of the biofilm dry weight, depending on biofilm type and conditions, and has important structural functions, which involve biofilm cohesion and mechanical stability due to electrostatic interactions with positively charged matrix constituents, acting as a horizontal gene transfer vehicle and antimicrobial peptide sequestrant, increasing biofilm tolerance. The sources of eDNA can be bacterial autolysis, active secretion, and neutrophil extracellular traps (NETs) that are released to combat infection, and the presence of NETs in subgingival periodontal biofilms of periodontitis patients. The structural significance of eDNA and its therapeutic potential may be supported by the ability of enzyme degradation of oral biofilms and its dispersal [24].

## *LIPID AND LIPOPOLYSACCHARIDE COMPONENTS*

Components of lipid and lipopolysaccharides (LPS) form a significant yet quantitatively minor part of the oral biofilm matrix compared to the polysaccharides and proteins, and the composition of the LPS is different between supragingival and subgingival biofilms. Gram negative species (*Porphyromonas gingivalis*, *Fusobacterium nucleatum*, and *Prevotella* spp.) release LPS in gram negative biofilms in their outer membranes, which, together with outer membrane vesicles (OMVs) lose into the outer membrane, supply important lipid and glycolipid components, which are incorporated into the extracellular matrix. These anionically charged molecules combine with matrix polysaccharides, proteins and divalent cations to facilitate matrix cohesion and also serve as strong pro-inflammatory pathogen-associated molecular patterns (PAMPs) to stimulate host immune response through Toll like receptors in periodontal tissues. Lipids (bacterial membrane lipids, LPS of minor Gram-negative constituents and host derived lipids) often constitute around 10-15% of the organic matrix in cariogenic supragingival biofilms, where they affect hydrophobicity and diffusion and mechanical behavior, but exopolysaccharides are still the major structural component [23].

## *MATRIX FUNCTIONS IN BIOFILM PATHOGENESIS*

The biofilm matrix is essential in biofilm pathogenicity which has a variety of mechanisms. The matrix offers primary adhesion binding loci in the form of glucan and other polysaccharide receptors, which allow primary bacterial adhesion and further biofilm formation. The matrix serves as a 3-dimensional framework that assembles the biofilm structure and, at the same time, facilitates biofilm integrity, keeping the multispecies

communities in tight contact with each other such that they can experience interdependence of their metabolic activity and polymicrobial synergy [25].

Biofilm matrix has a dynamic role in the antimicrobial tolerance and enamel demineralization instead of a passive scaffold. It forms diffusion limiting micro-environments which inhibit penetration and effective concentration of agents like chlorhexidine, fluoride and antibiotics, thus biofilm embedded bacteria develop significantly increased resistance in comparison to planktonic cells. The polysaccharide, eDNA, protein, lipid, and lipid-based network of negatively charged EPS binds and retards the incoming cationic antimicrobials, which enables even exposed multispecies biofilms sustained over high concentrations of chlorhexidine to recover. This type of 3D architecture preserves acidic focal points at the biofilm-enamel interface by preventing the efflux of acid as well as the influx of hydroxyl ions in the cariogenic biofilms, which is crucial in maintaining the pH at levels not permissible to remineralization and supporting enamel demineralization [26].

## BACTERIAL COMMUNICATION AND COORDINATION

### *QUORUM SENSING MECHANISMS*

Bacterial quorum sensing is a cell-density dependent signaling pathway that allows bacteria to carry out collective behavior with regard to population density. Quorum sensing is the process of producing diffusible signaling molecules (autoinducers) that are released into the culture supernatants; when the required concentrations are reached, the molcules bind the receptor proteins, causing widespread changes in gene expression and physiological behavior [27].

### *AUTOINDUCER-1 (AI-1) SYSTEMS*

Oligopeptide-based AI-1 systems are used by gram-positive bacteria such as Streptococcus mutans to communicate with each other within the species. Autoinducer peptides are generated as precursors which are protein processed through proteins and recognized by the two-component regulatory systems, such as the serine/threonine kinase and response regulator proteins. In S. mutans, AI-1 system controls the biofilm formation, bacteriocin production, competence development and expression of virulence factors [27].

*Porphyromonas* gingivalis has a system of AI-1 that controls the expression of virulence factors and the formation of biofilms. Recent findings indicate that *P. gingivalis* AI-1 signals mediate interactions between *P. gingivalis* and other species that include *Streptococcus mutans* in mixed-species biofilms, and thus functions of AI-1 are extended beyond intraspecies communication, suggesting interspecies signaling.

### *AUTOINDUCER-2 (AI-2) SYSTEMS*

The signal molecule that is involved in interspecies communication between Gram-positive and Gram-negative bacteria is autoinducer-2 (AI-2), a furanone-based signal molecule. The production of AI-2 is catalyzed by the LuxS enzyme (autoinducer-2 synthase), and the LuxS activity is controlled by the metabolism of methionine. The cross-kingdom communication coordinating the multispecies biofilm behavior is possible due to the ubiquitous nature of AI-2 signaling in the oral bacterial species [27].

*P. gingivalis* AI-2 signaling promotes cooperative interactions with other periodontal pathogens in oral biofilms, through an alteration of biofilm architecture and virulence. The nutritional dependencies that define synergistic pathogenicity between *P. gingivalis* and *T. denticola* are regulated by AI-2 signaling. Likewise, *S. mutans* AI-2 signaling is an interaction in multispecies biofilms in caries.

### *BIOFILM REGULATION GENES AND ENVIRONMENTAL SENSING*

The ComCDE quorum-sensing pathway in *Streptococcus mutans* links cell density with virulence by the competence -stimulating peptide (CSP) to signal the ComD/ComE two-component system, which subsequently enhances bacteriocin production, genetic competence, stress responses, and normal 3-D biofilm structure. Interventions with comC, comD, or comE lead to the appearance of uncharacteristic and low-biomass biofilms, which underscores ComCDE as an important regulator of *S. mutans* biofilm formation and pathogenicity [28].

LuxS/AI 2 is a highly conserved quorum -sensing system that regulates biofilm growth, carbohydrate catabolism, stress resistance, and the expression of virulence factors in *S. mutans* and other oral bacteria. LuxS mutation disrupts AI -2 production, results in compromised biofilms dependent on sucrose and Abnormal glucosyltransferase expression, and attenuates mixed-species biofilms with *Streptococcus gordonii* and *Porphyromonas gingivalis*, making LuxS/AI -2 a key virulence factor and a possible anti-biofilm agent [29].

### *QUORUM SENSING AND HOST INTERACTIONS*

Quorum sensing (QS) signals are now accepted to be two active mediators which regulate microbial behavior, as well as host responses. QS molecules generated by oral and

other bacteria are capable of being sensed by the epithelial cells, neutrophils and macrophages among other immune cells and modulate cytokine production, barrier properties and cell survival in a manner that may either amplify or suppress inflammation depending on the context [27].

N acyl homoserine lactones (AHLs), traditionally connected to Gram-negative bacteria, and AI 2 type signals can activate pattern recognition and danger sensing pathways in host cells, such as Toll like receptors (TLRs), NOD like receptors and inflammasome components, which result in the activation of NF κB, MAPK signaling and IL1β/IL18 processing. There is experimental evidence that AHL exposure can regulate the expression of pro inflammatory mediators (e.g. IL6, IL8, TNFα), affect epithelial barrier integrity and alter phagocyte activity, suggesting that QS signals are inter kingdom communication molecules that control the outcome of oral and systemic hostpathogen interactions instead of coordinating the behavior of bacterial groups [27].

## HOST IMMUNE RESPONSE AND INFLAMMATION

### *COMPONENTS OF ORAL INNATE IMMUNITY*

Elements of oral innate immunity constitute a layered primary line of defense that is constantly surveilling and acting against biofilm communities of the mouth. Innate and adaptive pathways are used in conjunction to identify pathogenic organisms and to generate inflammatory responses when required, and to limit the growth of microorganisms, although innate mechanisms offer the quick, nonspecific reaction that can take minutes to hours [5]. Physical barriers entail intact oral epithelium having tight junctions, the keratinized gingival epithelium and uninterrupted salivary flow, which mechanically removes microbes and dilutes soluble virulence factors. Saliva also has mucins and agglutinins which trap and cluster bacteria making their elimination easier by swallowing instead of mucosa or teeth being stuck [30].

Salivary and GCF humoral intrinsic factors are lysozyme, lactoferrin, peroxidase systems, complement components, antimicrobial peptides (defensins, cathelicidins), and all of which cause direct damage to bacterial membranes, chelate essential iron, or opsonized microbes to be phagocytosed. Phagocytic clearance, release of reactive oxygen species and proteases and antigen presenting to T and B cells are provided by cellular components, in particular neutrophils, macrophages, and dendritic cells recruited via the junctional epithelium, facilitating an interface between innate and adaptive responses to pathogenic biofilms [2].

### *SALIVA AND MUCOSAL BARRIER FUNCTION*

Saliva also performs several antimicrobial roles such as mechanical cleansing, acid buffering of food and bacterial organic acids, remineralization enhancement by calcium and phosphate transport and antimicrobial proteins and peptides. Salivary immunoglobulins, such as secretory IgA avails immune exclusion against pathogenic biofilm adhesion. Salivary lysozyme has bacteriolytic activity on many organisms. Lactoferrin has iron-withholding antimicrobial activities. Salivary histatin antimicrobial peptides have antifungal and antibacterial effects [27].

The junctional epithelium forms a critical physical and immunological barrier at the gingival sulcus, where tight junction proteins and specialized cell–cell contacts help maintain epithelial integrity and restrict bacterial invasion into underlying connective tissues. Pattern recognition receptors are expressed by oral epithelial cells, including Toll -like receptors (TLRs) and NOD -like receptors (NLRs) that perceive pathogen-associated molecular patterns and trigger downstream signaling cascades (e.g., NF- -B, MAPK), which result in the production of antimicrobial peptides and release of pro-inflammatory mediators which coordinate innate and adaptive immune responses to dental biofilms [2].

### *NEUTROPHILS AND INNATE CELLULAR RESPONSE*

Neutrophils (polymorphonuclear leukocytes) constitute the major cellular constituent of innate inflammation of the oral tissues. They are found in gingival crevicular fluid and periodontal pockets, activated by biofilm-generated chemotactic factors and complement activation. Neutrophils infiltrate periodontal tissues in a low concentration on a regular basis, which is a homeostatic state that occurs under steady-state conditions and that actively involves antimicrobial activity and tissue support [31].

Neutrophil antimicrobial mechanisms include: (1) phagocytosis - internalization and intracellular degradation of pathogens through fusion of phagolysosomes containing hydrolytic enzymes and antimicrobial proteins; (2) degranulation - extracellular release of granule contents including elastase, lactoferrin, lysozyme, and antimicrobial peptides; (3) reactive oxygen species (ROS) production—generation of superoxide, hydrogen peroxide, and other reactive species directly toxic to microbes; and (4) neutrophil extracellular trap (NET) formation - release of decondensed chromatin decorated with histones and granule contents entrapping microorganisms [31].

Neutrophil extracellular traps (NETs) have become a significant immune response that plays two roles. The antimicrobial proteins and peptides in NETs increase

local antimicrobial response against periodontal pathogens. Nevertheless, excessive NET leading to the buildup of the host tissue is associated with the release of damaging enzymes such as neutrophil elastase, collagenase, as well as other enzymes that degrade the matrix. There is a role of NET-derived enzymes in the alveolar bone loss in progressive periodontitis.

## *INFLAMMATORY CYTOKINES AND CHEMOKINES*

Bacterial proteins, lipopolysaccharides, and peptidoglycans produced in biofilms activate epithelial cells and macrophages to release pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-1- beta (IL-1β), and interleukin-6 (IL-6). These cytokines amplify the effects of inflammatory responses in several ways, such as augmenting neutrophil recruitment, augmenting the vascular permeability as well as augmenting the expression of adhesion molecules [2].

Chemokines, including IL-8, MCP-1 and RANTES coordinate selective leukocyte transport to the locales of biofilm triggered inflammation, with an enhancement in MCP 1 and RANTES concentrations in gingival crevicular fluid and tissues positively associated with probing depth and clinical attachment loss in periodontitis. It is also worth noting that *Treponema denticola* expresses a surface protease dentilisin and a complex of a chymotrypsin like protease, which can degrade host inflammatory mediators, such as IL1β, IL6, TNFα, and a number of chemokines, to disrupt normal neutrophil and monocyte recruitment and lead to a dysregulated inflammatory environment and dysbiosis in periodontal epithelial pockets [32].

## *MATRIX METALLOPROTEINASES AND TISSUE DESTRUCTION*

MMPs are major mediators of periodontal tissue destruction and include a family of zinc and calcium-dependent endopeptidases that cleave extra-cellular matrix components (fibrillar collagens (type I, II, III), elastin, gelatin, laminin, fibronectin, and proteoglycans). The neutrophil collagenase (MMP-8) and interstitial collagenase (MMP-1) activated by periodontitis trigger the breakdown of periodontal ligament type I collagen at select Gly-Ile/Leu bonds to form collagen telopeptides cleaved by gelatinases MMP-2 and MMP-9 to peptides. MMP-13 (collagenase-3) is also increased in pathologic gingivae and leads to bone erosion [33].

Periodontitis MMP activation is a process with bacterial and host pathways. *Porphyromonas gingivalis* gingipains (RgpA/B, Kgp) are a direct proteolytic activator of latent pro-MMP-9/2 and pro-MMP inhibitors, and induce more extensive matrix destruction. Host pro-inflammatory cytokines (TNF-α, IL-1β, IL-6) induce MMP-1, MMP-3, MMP-8, MMP-9, and MMP-13 expression in fibroblasts, macrophages, and gingival epithelial cells via NF-κB and AP-1 signaling. Bacterial LPS and other PAMPs further upregulate MMP production through TLR signaling

In physiological conditions, MMP activity is under the strict control of tissue metalloproteinase inhibitors (TIMPs 1 4 ), which are able to form 1:1 metal protease active complexes with active MMPs to keep extracellular matrix homeostatic. In periodontitis, net proteolytic unbalance supporting tissue destruction, an increase in the levels of MMP-8 and MMP-9 in gingiviable crevicular fluid and diseased tissues are always accompanied by MMP overexpression and relative deficiency of TIMP (especially TIMP-1 and TIMP-2) [2].

## *ADAPTIVE IMMUNE RESPONSE*

Immunogenicity of oral pathogens is best characterized as adaptive immunity that is partially protective. Periodontal pathogen specific antibodies Periodontal pathogen specific antibodies (primarily IgA in saliva and IgG in serum) can be detected in caries and periodontitis respectively, respectively, in individuals with antigen mediated humoral responses. Increased salivary anti s. mutans IgA may be related to reduced caries experience in certain cohorts, but in most periodontal and caries research, high levels of pathogen specific antibody are related to disease presence or disease severity rather than protection, which may indicate that antibody production is often a measure of persistent antigenic stimulation and ineffective immune control as opposed to the presence of optimal sterilizing immunity [34].

Tcell responses in periodontal disease involve multiple $CD4^-$ Thelper subsets with distinct effector profiles. Th1 cells produce IFNγ and TNFα and support cellmediated immunity; Th2 cells secrete IL4 and IL10 and favor humoral responses; Th17 cells produce IL17 and IL22, driving neutrophil recruitment, osteoclastogenesis, and proinflammatory cytokine cascades; and regulatory T cells (Tregs) expressing FOXP3 secrete IL10 and TGFβ to restrain excessive inflammation. In periodontitis, an imbalance characterized by increased Th1/Th17 activity and reduced Th2/Treg function is consistently associated with heightened inflammatory mediator levels and alveolar bone loss, and Th17/Treg ratios in blood and periodontal tissues correlate with disease severity and response to therapy. More recently, γδ T cells have emerged as important regulators of gingival immunity: oral barrier γδ T cells can either limit or exac-

erbate periodontal pathology depending on context, contributing to tissue repair via amphiregulin production but also promoting bonedestructive responses in some infection models, highlighting their dual role in chronic periodontal inflammation [2].

## PREVENTION STRATEGIES

### *ORAL HYGIENE AND MECHANICAL PLAQUE REMOVAL*

Toothbrushing and interdental cleaning are the bases of caries and periodontal disease prevention as they remove plaque, which is entirely mechanical. Removal of pathogenic biofilms before they become pathogenic consortia of pathogenic bacteria by means of daily plaque removal through brushing of the teeth. Proper fluoridated toothpasting with fluoride concentrations of 1000 ppm fluoride or higher reduced caries incidence significantly, and there is evidence that it is effective, especially in fluoride levels of 1000-1500 ppm fluoride (to prevent caries) and 5000 ppm fluoride (to protect high-risk individuals)[35].

Interdental cleaning (floss, interdental brushes, wood sticks, oral irrigators) is required to access interproximal and subgingival spaces that can not be reached by toothbrush bristles and it can prevent proximal caries and interproximal periodontal disease. Systematic and scoping reviews show that interdental devices have weak to moderate adjunctive effects in the control of plaque and gingivitis, with interdental brushes demonstrating more consistent results than floss due to the restricted nature of the evidence and the significant effect of compliance and technique on results.

### *FLUORIDE APPLICATION AND MECHANISMS*

Fluoride exhibits multiple anti-caries mechanisms: (1) inhibition of demineralization through fluorapatite formation, which is more acid-resistant than hydroxyapatite; (2) enhancement of remineralization through deposition of fluoride-containing minerals; (3) inhibition of bacterial glycolytic enzymes, including enolase and lactate dehydrogenase, thereby reducing acid production and bacterial growth [35].

Some of the modalities of delivering fluoride are through water fluoridation, fluoride toothpaste, mouthrinses, professionally applied gels and varnishes, and fluoride supplements in the diet. The efficacy of 0.7-1.0 ppm in water fluoridation is that it virtually eliminates caries by a margin of 25-30 percent among the population with sufficient water fluoridation. There is moderate-level evidence in the use of professionally administered fluoride gels (1% sodium fluoride) and varnishes (22,600 ppm) as caries prevention methods. Recent systematic reviews show that professionally applied fluoride varnishes have clinical benefit in caries prevention, as resin-based fissure sealants have the same or even higher effectiveness in caries prevention as fluoride varnish applications.

### *PIT AND FISSURE SEALANTS*

Dental sealants (resin-based and glass ionomer) are physical barriers to cover occlusal pits and fissures of posterior teeth, inhibiting the accumulation of plaque and inhibiting the access of nutrients to fissure microorganisms. The majority of caries in children and adolescents happens on the occlusal surfaces, on which the fissure anatomy is slender and complex, making the brush bristles of the toothbrush inaccessible; thus, sealants are effective among primary and permanent molars.

There is high-grade evidence indicating that pit-and-fissure sealants can significantly prevent the incidence and progression of caries in the pit-and-fissure area compared with no sealant or fluoride varnish alone, with the guideline panels indicating that the risk is reduced by about 70-80 percent over 2-3 years in children and adolescents. Modern reviews find that resin-based sealants offer better retention and caries prevention effect where adequate isolation and moisture control is possible, but glass ionomer sealants, though with low retention, are more beneficial in partially erupted molars or where moisture control cannot be adequately controlled, and may be equally effective in the long term prevention of caries in those situations.

### *DIETARY MODIFICATION AND CARBOHYDRATE RESTRICTION*

Dietary change and carbohydrate restriction contain the risk of caries by reducing the number and severity of biofilm acid challenges. Frequent consumption of free sugars, especially sucrose in snacks and beverages between meals, is strongly associated with higher caries activity in children, adolescents, and adults, whereas limiting free sugars to less than 10% (ideally <5%) of total energy intake and restricting sugar exposures to ≤3–4 times per day substantially reduces caries risk. Frequent consumption of sugary snacks by high frequency raises the odds of caries several folds as compared to low frequency, and epidemiologic evidence indicates consistently that the frequency and quantity of sugar consumption, especially outside of mealtime, is a crucial factor in caries experience [35].

On the biofilm level, sucrose is the only cariogenic fermentable material since it can be used as a fer-

mentable material to produce acids, and it can be used as an extracellular polysaccharide material to produce EPS-rich, acidogenic, and aciduric biofilms. Decreasing the amount of carbohydrate intake, reducing the amount of sucrose and refined carbohydrates reduces substrate availability to *Streptococcus mutans* and other cariogenic organisms, decreases EPS production and biofilm acidogenicity, and restricts ecological selection of acid-tolerant species. Substituting sugar-free alternatives - particularly xylitol- or sorbitol-based chewing gums and confections - further reduces cariogenic potential, as polyols are poorly fermented by plaque bacteria and can stimulate salivary flow; multiple clinical trials and meta-analyses show that regular xylitol gum use decreases caries incidence and *S. mutans* levels compared with sugared controls, especially when used several times daily after meals [35].

## *XYLITOL AND SUGAR ALCOHOL CARIES PREVENTION*

Xylitol, a naturally occurring five-carbon sugar alcohol, exhibits caries-preventive properties through multiple mechanisms: (1) nonfermentability by oral bacteria - xylitol is scarcely metabolized by cariogenic microorganisms, preventing fermentation and acid production that drive enamel demineralization; (2) antimicrobial effects through metabolic disruption - when *Streptococcus mutans* takes up xylitol, it is phosphorylated but not further metabolized, creating a futile cycle that depletes cellular energy, inhibits bacterial growth, and reduces acid and virulence factor production; (3) reduced extracellular polysaccharide synthesis by *S. mutans*, thereby decreasing plaque adhesiveness and biofilm accumulation; (4) saliva stimulation through mastication, enhancing saliva-mediated remineralization, pH buffering, and antimicrobial clearance; and (5) microbial selection toward less cariogenic xylitol-resistant *S. mutans* strains with attenuated virulence, although the long-term clinical significance of this shift is still being clarified [36].

Meta-analysis demonstrates xylitol reduces DMF/dmf (decayed, missing, filled surfaces/teeth) scores with a standard mean reduction of -1.09 compared to all controls, and -1.87 reduction compared to fluoride varnish controls [36]. Newer systematic reviews of xylitol versus other polyols like sorbitol and erythritol reveal that xylitol lowers caries increment and *S. mutans* counts, and erythritol may have potentially better efficacy in some long-term studies, but the clinical implications of the differences have not yet been studied. In particular, erythritol showed inferior and slower caries formation relative to xylitol and sorbitol during a 3 year follow-up type study in child groups, and had reduced dental treatments.

Xylitol can be found in various products, which are chewing gum, lozenges, mints, candies, syrups, toothpastes, etc. American Academy of Pediatric Dentistry recommends xylitol 3-8g/day in the form of syrup or wiping to children at ages below 3 years; and age-specific products like gum or candies to children aged 4 and above years. Among those who are at risk of cavities (adolescents and adults), and those with high caries rates, clinical evidence indicates that a 5-10g/day, 3-5 times regimen is the most effective regimen in preventing caries incidence.

## *ANTIMICROBIAL AGENTS AND CHLORHEXIDINE*

Chlorhexidine (CHX) is a cationic biguanide that has been shown to have a broad-spectrum antimicrobial activity and a high activity against both aerobes and anaerobes. The CHX antimicrobial action is based on attraction of the positive charge to negatively charged bacterial cell surfaces, which results in the strong adsorption on the bacterial cell membranes, cell wall disruption, cytoplasmic leakage, and bacterial death at increased concentration. CHX demonstrates substantivity, i.e. antimicrobial activity remaining after as long as 12 hours after application, by retention on oral surfaces such as tooth enamel, mucosa and salivary glycoproteins.

Chlorhexidine mouthwash at 0.12% concentration demonstrates superior efficacy for plaque control; rinsing for 60 seconds twice daily with 10 ml of chlorhexidine inhibits plaque growth by approximately 60% and reduces gingivitis by 50-80%. Clinical use of chlorhexidine in chronic prevention is however, limited because of the side effects such as tooth discoloration, taste disorder and mucosal erosions. The existing knowledge underlies short-term use (weeks to several months) of chlorhexidine in specific indications, not in chronic prevention.

It is worth noting that exposure to chlorhexidine causes microbiota changes that can lead to disease-related dysbiosis in certain instances. Exposure of biofilm to chlorhexidine leads to initial microbial inactivation and a rapid increase in biomass with altered microbial composition, in certain cases with an increase in the abundance of pathobiont strains and a shift in metabolic activity towards disease-associated lactate production.

## *PROBIOTICS AND PREBIOTICS*

Probiotics are live microorganisms that, when ingested in sufficient quantities, provide a health benefit to the

host and have been explored as adjunctive measures, not as treatment in themselves, in the prevention of caries and periodontitis. Competitive exclusion, co-aggregation as well as secretion of antimicrobial compounds including organic acids, hydrogen peroxide and bacteriocin-like peptides along with the regulation of local immune responses can inhibit the growth and virulence of cariogenic and periodontopathogenic species by oral probiotic strains like *Lactobacillus reuteri*, *Streptococcus salivarius* (K12, M18) and various Bifidobacterium species [37].

In oral health, probiotic mechanisms are better framed as:

1. Competing with pathogens for adhesion sites and nutrients on oral surfaces and within biofilms, and, as a result, colonization by cariogenic and periodontal pathogens [37]
2. Instead of preventing the formation of calculus, producing antimicrobial metabolites (e.g. bacteriocins, hydrogen peroxide, organic acids) that are able to inhibit biofilm formation and modify biofilm composition
3. Modulating inflammatory responses by down-regulating pro-inflammatory cytokines such as IL-1β, IL-6, TNF-α, and MMP-8 and enhancing anti-inflammatory mediators like IL-10 and TIMP-1
4. Enhancing tissue remodeling and periodontal stability by decreasing the burden of inflammation and changing the microbiota composition to a less pathogenic state indirectly.

Recent clinical trials prove that periodontal disease clinical markers, such as bleeding on probing and probing pocket depth, are decreased by probiotics in chewing gum or in lozenges. A meta-analysis of the effectiveness of probiotics in the management of periodontitis suggests that probiotics decrease periodontal inflammatory (IL-1β, MMP-8) and improve the decrease in probing pocket depth and gain in clinical attachment level.

Prebiotics are non-digestible substances that are selectively used by host microorganisms with a health effect; in the mouth, inulin -type fructans and fructo -oligosaccharides can stimulate growth of desirable lactobacilli and bifidobacteria and could aid in repositioning the oral microbiome to be less dysbiotic. Combined prebiotics add to the particular probiotics have been identified as synbiotics that seem to have improved efficacy due to synergistic effects, and the recent reviews and preliminary clinical trials indicate that synbiotic or postbiotic preparations can be more beneficial to caries outcomes in children and periodontal treatment response in adults compared to the individual components.

## TREATMENT APPROACHES

### *MECHANICAL DEBRIDEMENT: SCALING AND ROOT PLANING*

Scaling and root planing (SRP) is the gold standard non-surgical mechanical therapy of periodontal disease, and the central part of primary therapy in most patients with chronic periodontitis. SRP in periodontal therapy mechanically debridges tooth surfaces with supra- and subgingivally located biofilm and calculus, destabilizes organized biofilm communities, and smoothes root surfaces and, thus, reduces pathogenic biomass, enhancing periodontal healing. Even though mechanical debridement and plaque removal also feature in the caries control, SRP *per se* is instead chiefly established in terms of periodontal, not coronal, caries management.

SRP induces treatment responses in a number of ways, with direct destruction of bacteria by destabilizing biofilm structure, exposing anaerobic microorganisms to oxygen, which creates an unfavourable environment to obligate anaerobes, and by eliminating bacterial lipopolysaccharides and other pathogenic surface composites of root cementum. Such changes alter the subgingival environment, decrease inflammation, and on average have an average probing depth decrease of about 1-2 mm and clinical attachment improvements of about 0.5-1 mm based on initial pocket depth.

Nonetheless, there are significant drawbacks to SRP. The difficulty of removing 100 percent of calculus and biofilm is increasingly challenging in deep pockets, furcation sites and complex root morphologies, and research reveals that, even after closed SRP, 30-60 percent of the root surfaces might still have some residual subgingival calculus, particularly when the pocket depth is more than 6 mm. Clinically, an impressive percentage of locations or patients (usually estimated at about 20-40% of moderate-severe cases of chronic periodontitis) have residual deep pockets and evidence of ongoing inflammation following SRP alone and require adjunctive measures (usually considered surgical access, local or systemic antimicrobials, or photodynamic therapy).

### *SYSTEMIC ANTIBIOTICS*

A combination of adjunctive systemic antibiotics with SRP augments the therapeutic effect, especially in aggressive periodontitis and in chronic periodontitis patients with poor response to monotherapy when using SRP. Various combinations of antibiotics are effective, the most well-researched and clinically validated combinations being metronidazole (used or used with amoxicillin or azithromycin). Recent systematic reviews

affirm that amoxicillin, metronidazole, and azithromycin offer the most uniform extra benefits in probing depth reduction and clinical level of attachment, particularly in deep pockets and in the progressively advancing disease.

The antibiotic efficacy mechanisms include: decreasing obligate anaerobic periodontal pathogens by direct antimicrobial attack, altering polymicrobial synergism by selective destruction of critical species, and attenuation of bacterial virulence factor expression. Systemic antibiotics combined with SRP show a mean additional clinical attachment gain of between 0.2-0.4 mm and a probing depth reduction of between 0.3-0.6 mm on average compared to SRP monotherapy, with the greatest effect reported with amoxicillin-metronidazole combinations. The positive effects are seen in the aggressive periodontitis patients, where the mean gain of attachment is significantly higher than in chronic periodontitis, especially in stage III/grade Cs.

Nevertheless, developing antimicrobial resistance is a severe disadvantage of long-term or repeated systemic antibiotics, and emerging resistant pathogens diminish clinical efficacy with time and represent a more general public-health threat. Recent umbrella reviews have determined that, whilst statistically significant improvement is obtained with adjunctive systemic antibiotics, the effect size on a population level is small, and it is not justified to use this in all periodontitis cases. Recent evidence-based recommendations and guidelines encourage a restrictive, case-by-case approach and administration of systemic antibiotics only on specific high-risk patients or non-responsive patients, with short and well-defined courses of antimicrobials in coordination with completion of SRP and focus on concomitant mechanical debridement and antimicrobial mouthrinses to reduce the duration of treatment and risk of resistance.

## *ANTIMICROBIAL PEPTIDES*

Antimicrobial peptides (AMPs) are innovative therapeutic tools that have a strong anti-oral biofilm activity and a low tendency to develop resistance to them due to their fast and multitarget effects on bacterial membranes and intracellular elements. Peptide 1018 (IDR-1018) is a synthetic cationic amphiphilic peptide, the analogue of bovine host-defense peptide bactenecin, with a broad-spectrum antibiofilm activity against oral bacteria such as Streptococcus mutans, Enterococcus faecalis and mixed-species oral biofilms at significantly lower concentration levels than its MIC values against planktonic cells [38].

Mechanisms of peptide 1018 triggering the ability to induce biofilm cell death by targeting and inducing the degradation of the nucleotide second messenger (p) ppGpp, a central regulator of the stringent response and the survival of biofilms, and cause cell lysis and disruption of the biofilm structure as observed under electron microscopy. The peptide disperses and kills preformed biofilms as well as prevents biofilm formation, and there are large changes in viable biofilm bacteria at sub-MIC concentrations.

Notably, peptide 1018 does not lose its antibiofilm capability when combined with saliva, which is also clinically significant because, in vivo, most AMPs will be inactivated by salivary and bacterial proteases in the oral cavity. The combination treatment of peptide 1018 at low-concentration chlorhexidine (2%) significantly increases the efficacy of antibiofilm treatment, killing biofilm by greater than 50 and dispersing biofilm by more than 35 of 3-minute exposure in multispecies oral biofilm models.

Human β-defensin-3 (HBD-3) demonstrates enhanced bactericidal activity against mixed-species oral and endodontic biofilms compared to conventional agents such as calcium hydroxide and chlorhexidine, particularly against *E. faecalis* within dentinal tubules. HBD-3 synthetic HBD 3 derivatives (e.g. HBD3 -C15) have been demonstrated to be more penetrative and kill biofilm bacteria in dentin models and can act synergistically with traditional irrigants to promote HBD3 -based formulations to be used in endodontic and periodontal practice.

## *PHOTODYNAMIC THERAPY*

The antimicrobial photodynamic therapy (aPDT) is an academic technique of treatment using light irradiation in combination with a photosensitizer molecule to produce reactive oxygen species (ROS) that are harmful to microorganisms, such as singlet oxygen. aPDT is a non-invasive method that requires interaction of a photosensitizer, molecular oxygen, and visible light to create reactive oxygen species (ROS). The treatment is usually administered as a topical photosensitizer (usually indocyanine green -ICG) application and later irradiation of the skin with lasers with certain wavelengths corresponding to the spectral absorptions of photosensitizers. Indocyanine green combined with a diode laser of 810 nm produces singlet oxygen and the effect of other reactive oxygen species with bactericidal activity against periodontal pathogens. The process entails the electron transfer under the influence of a photon via Type II photosensitization where ICG causes intense cellular damage when exposed to a near-infrared-light. The produced singlet oxygen quickly interacts with the bacterial biomolecules, which results in cross-

linking lipids of the membrane, damage to proteins and ion channels, and the removal of metabolic enzymes.

A clinical trial has shown that aPDT when used as an adjunct to scaling and root planing (SRP) and laser-assisted is very effective in reducing probing pocket depth (PD) along with increasing clinical attachment level (CAL) over SRP used alone. Repeated applications of aPDT significantly improved outcomes, reducing residual pockets greater than 5 mm by more than 40%, with effects more pronounced in deep sites (PPD ≥ 6 mm) [39].

aPDT demonstrates efficacy against periodontal pathogens, including *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans*, *Fusobacterium nucleatum*, and *Tannerella forsythia*, with bacterial load reductions of 95-99%. Beyond bacterial killing, aPDT promotes healing through immunomodulatory effects by suppressing inflammatory mediators such as TNF-α and IL-1β, resulting in decreased inflammation and enhanced tissue regeneration.

The benefits associated with the use of eye products are non-development of antibiotic resistance, non-invasive use, and possible repetitive use without the occurrence of resistance. The multi-target mode of action also results in less resistance, unlike antibiotics, since the ROS floods antibacterial antioxidant responses at the same time. It has been proposed through comparative studies that aPDT is an effective alternative to systemic antibiotics in the treatment of periodontitis. The existing evidence confirms the efficacy of aPDT as an adjunctive therapy, but concerns the cost-efficiency and the best protocols. Studies on photosensitizers differ significantly in the choice of treatment regimens, laser settings, and frequency of use. When using multiple applications (2-4 sessions), the results are superior to single applications. It is justified that future standardized clinical studies involving larger cohorts with a longer follow-up period should be conducted to develop some standard guidelines.

## PROBIOTICS AS ADJUNCTIVE THERAPY

New data are in favor of probiotics as a complement to traditional periodontal therapy. The mechanistic explanation includes the replacement of pathogenic species by the competitive exclusion, disruption of biofilms by antimicrobial compounds production, and the immune modulation in favor of healing. Clinical trials prove the use of *Lactobacillus reuteri* strains integrated into chewing gum when using it together with the traditional SRP lead to better clinical parameter changes such as the decrease of bleeding on probing, the decrease of probing pocket depth, and the improvement of the clinical level of attachment in comparison with SRP and placebo [40].

# CONCLUSIONS

Clinical trials prove that the use of *Lactobacillus reuteri* strains integrated into chewing gum, when used together with the traditional SRP lead to better clinical parameter changes, such as the decrease of bleeding on probing, the decrease of probing pocket depth, and the improvement of the clinical level of attachment in comparison with SRP and placebo.

There are more than 500 different types of bacteria in the oral microbiota that remain in symbiosis in a state of health. The selective growth of disease-causing consortia such as Streptococcus mutans and related acid-tolerant pathogens in caries, and the red complex (*Porphyromonas gingivalis, Treponema denticola, Tannerella forsythia*) in periodontal disease are dysbiotic changes to disease. These pathogens have complex virulence factors such as biofilm formation, production of extracellular polysaccharides, synthesis of proteolytic enzymes as well as immune evasion mechanisms that enable pathogenesis even in the presence of strong host immunity.

The formation of biofilms is the key pathogenic process in both pathologies, and the extracellular polymeric substance matrix offers key scaffolds, diffusion-restricting microenvironment, and antimicrobial resistance. Bacterial communication Quorum sensing, regulates the expression of virulence factors and biofilm formation in multispecies communities and creates regulatory mechanisms to switch between commensal existence and pathogenic phenotype.

The host immune responses to biofilm challenge include innate responses such as neutrophil recruitment and production of matrix metalloproteinase and adaptive responses (antibody and T cell). Although the roles of these immune processes are protective, overreactivity or malregulation of these mechanisms, especially neutrophil and MMP activation, is involved in the processes of destructive periodontal tissue destruction that accelerates the onset of the disease.

Evidence-based prevention strategies are mechanical plaque removal, fluoride application, dietary modification, and emerging ones that involve the use of antimicrobial agents that have lower resistance potential. The paradigms of treatment are still developing past the traditional use of mechanical debridement and antibiotics to modality specific approaches such as antimicrobial peptides, photodynamic therapy and antibiotic-based approaches based on dysbiotic restoration of oral microbiota.

Future research should prioritize: (1) identification of dysbiosis markers enabling early disease intervention; (2) development of interventions specifically targeting keystone pathogens and dysbiosis reversal rather than

non-specific biofilm removal; (3) integration of omics technologies providing systems-level understanding of host-microbe interactions; (4) development of drug delivery approaches improving therapeutic agent penetration into biofilm microenvironments; and (5) personalized treatment approaches incorporating individual host susceptibility factors and microbiota profiles guiding intervention selection.

The microbiology of dental caries and periodontal disease has also been undergoing advanced development with complex molecular applications and systems thinking. The increased knowledge provides the possibility of rational, mechanism-based intervention development that enhances the effectiveness of disease prevention and treatment and minimizes the antimicrobial resistance and other adverse effects of traditional methods.

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**CONFLICT OF INTEREST**

The authors declare no conflict of interes

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