Chapter 2

# Impact of Dental Plaque Biofilms in Periodontal Disease: Management and Future Therapy

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### Abstract

Oral cavity represents an ideal environment for the microbial cell growth, persistence, and dental plaque establishment. The presence of different microniches leads to the occurrence of different biofilm communities, formed on teeth surface, above gingival crevice or at subgingival level, on tongue, mucosa and dental prosthetics too. The healthy state is regulated by host immune system and interactions between microbial community members, maintaining the predominance of "good" microorganisms. When the complexity and volume of biofilms from the gingival crevice increase, chronic pathological conditions such as gingivitis and periodontitis can occur, predisposing to a wide range of complications. Bacteria growing in biofilms exhibit a different behavior compared with their counterpart, respectively planktonic or free cells. There have been described numerous mechanisms of differences in antibiotic susceptibility of biofilm embedded cells. Resistance to antibiotics, mediated by genetic factors or, phenotypical, due to biofilm formation, called also tolerance, is the most important cause of therapy failure of biofilm-associated infections, including periodontitis; the mechanisms of tolerance are different, the metabolic low rate and cell's dormancy being the major ones. The recent progress in science and technology has made possible a wide range of novel approaches and advanced therapies, aiming the efficient management of periodontal disease.

**Keywords:** dental plaque biofilm, periodontitis, host defense mechanisms, resistance mechanisms, therapeutic approaches

## 1. Introduction

Oral cavity represents an ideal environment (e.g., appropriate temperature and nutrients) for the microbial cell growth, survival and persistence, and subsequent dental plaque biofilm establishment. The exact number of species from the oral plaque is not known, because some of them are not cultivable, but it is estimated to be between 700 and 1000 species, reaching densities of $10^8$ bacterial cells/mg, much of them being uncultivable [1]. However, bacteria are the most numerous group in the oral microbiota, accompanied by a diverse collection of archaea, fungi, protozoa, and viruses. The oral microorganisms are generally commensal species, maintaining relationships with the host based on mutual benefits. They do not produce disease, but instead impede pathogenic species to adhere to mucosal surfaces [2, 3].

Dental plaque biofilm represents a polymicrobial community that remains relatively stable in health, consisting in species belonging to *Streptococcus, Actinomyces, Veillonella, Fusobacterium, Porphyromonas, Prevotella, Treponema, Neisseria, Haemophilus, Eubacteria, Lactobacterium, Capnocytophaga, Eikenella, Leptotrichia, Peptostreptococcus, Staphylococcus*, and *Propionibacterium* genera.

The dental plaque biofilm formation follows many stages and begins at 1 h after washing, when the tooth surfaces are covered by an organic "pellicle" composed from salivary glycoproteins, carbohydrates and immunoglobulins, which are adsorbed on the hydroxyapatite surface through electrostatic interactions between calcium ions and phosphate groups with the oppositely charged groups of the macromolecules from the saliva. In a second stage, bacteria adhere to the pellicle and between them through the interaction between specialized structures or adhesins (glycocalyx, capsule, and fimbriae) with complementary receptors. The first colonizers are gram-positive cocci (*Str. mutans, Str. mitis, Str. sanguis* or *Str. oralis, Rothia dentocariosa,* or *Staphylococcus epidermidis*), gram-positive rods, actinobacteria (*Actinomyces israelis* and *A. viscosus*) and few gram-negative cocci [4]. The attached species secrete exopolymers such as glucans that contribute to the development of biofilm matrix and allow association of other species. Although initially the oral cavity offers an aerobic condition, oxygen is rapidly consumed by the aerobic bacteria (e.g., *Neisseria* spp.) or facultative anaerobic (e.g., *Streptococcus* and *Actinomyces* spp.), which are first colonizers creating appropriate conditions for the survival of obligate anaerobe species. When biofilm reaches maturity, the oral cavity becomes colonized predominantly by anaerobic bacteria [5].

In the oral cavity, the presence of different microenvironments leads to the occurrence of different biofilm communities, like those formed on the surface of teeth above the gingival crevice (the supragingival plaque) or at the subgingival level (the subgingival plaque), on the tongue, on the mucosal surfaces, or biofilms developed on dental prosthetics and fillings. Some microbial species are better adapted to some location. For example, based on their oxygen requirements, species could be classified as obligate aerobes, obligate anaerobes (as *Veillonella* and *Fusobacterium*), facultative anaerobes (as most streptococci and *Actinomyces*), and microaerophilic species that prefer low concentrations of $O_2$ (from 2 to 10%) and capnophilic (species that grow best at high $CO_2$ concentrations, from 5 to 10%, as *Neisseria*) [3].

When the complexity and volume of biofilms located in the gingival crevice increase, pathological conditions such as periodontitis or chronic gingivitis can occur. Literature of the last decades has shown that almost all forms of the periodontal disease are consequences of the chronic, nonspecific or specific bacterial infections. If in the healthy individuals, the oral biofilms are comprised mainly of gram-positive facultative anaerobes (*Streptococcus anginosus* and *A. naeslundii*), in the above mentioned pathologic conditions, the percentage of gram-negative anaerobic bacteria increases and may include *Aggregatibacter* (previously *Actinobacillus*) *actinomycetemcomitans, Porphyromonas gingivalis, P. intermedia, Bacteroides forsythus, Campylobacter rectus, Eikenella sp., Peptostreptococcus micros, Streptococcus intermedius, Prevotella sp., Fusobacterium sp., Capnocytophaga sp., Veillonella sp., Treponema* and other on-cultivable spirochetes, and the bacterial counts associated with the disease are up to 10(5) times larger than those of the same species found in healthy individuals [6, 7].

Perhaps, the three best-studied periodontal pathogens are *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans,* and, more recently, *Bacteroides forsythus*, all three carrying pathogenicity islands and having the ability to secrete a number of virulence factors, including invasion of gingival epithelial cells and an abundant array of extracellular proteases. The last ones are responsible for the increase in vascular permeability and in the flow of gingival crevicular fluid (GCF), thus providing a rich source of nutrients for the subgingival plaque community.

*Porphyromonas gingivalis* is one of the most important periodontal pathogens, exhibiting the ability to adhere and invade epithelial tissue of the oral cavity *in vitro*. *Aggregatibacter (A.) actinomycetemcomitans* is associated with periodontal disease in preteen ages. *Fusobacterium nucleatum* is an important periodontal agent, especially in the rapid and progressive periodontal disease forms. *Prevotella intermedia* is black-pigmented, while *Bacteroides (B.) forsythus* an unpigmented gram-negative bacterium; *B. forsythus* has several virulence factors, including the production of trypsin-like proteases and polysaccharides, the ability to penetrate the host cell, or inducing of apoptosis. *Capnocytophaga* species are involved in the onset of the juvenile periodontal disease and in the periodontal disease of adults. These bacteria produce pro-inflammatory lipopolysaccharides and extracellular proteases that could destroy sIgA immunoglobulins. Prevalence *of Peptostreptococcus micros* in advanced periodontitis in adults has been reported as 58–63%. It was also positively associated with dental implant failure. Spirochetes were observed in a greater proportion in patients with periodontal disease than in healthy individuals [8]. Two important spirochetes species, i.e., *Troponema vincentii* and *T. denticola,* are also involved in periodontal disease. Both produce pro-inflammatory lipopolysaccharides and unusual metabolic products, such as indole, hydrogen sulfide and ammonia that are potentially toxic to the host cells.

Besides the microbial component, genetic, physiological, and behavioral factors are also involved in the pathogenesis of periodontal disease. Some people may be genetically susceptible to periodontal disease, but the genetic background involved is not clear. The hormonal changes associated with teen age and pregnancy could contribute to gingival enlargement. Smoking is among the factors that increase the probability to develop a periodontal disease. In smokers, reduced gingival blood flow, impaired wound healing, and increased production of inflammation-mediating cytokines were observed comparing with healthy persons. Smoking seems to increase the severity of periodontal disease, but also the response of the gingival tissues to periodontal therapy is reduced, fact that contributes to a greater incidence of refractory disease and to the risk to lose teeth. Regarding age, the researches indicate that older people have the highest rates of periodontal disease. Other factors which may contribute to evolution of periodontitis are diet, stress, obesity, and some other underlying diseases such as diabetes, cardiovascular disease, osteoporosis, and rheumatoid arthritis. Certain medications could also be inappropriate for the evolution of periodontal disease. Also, a bad oral hygiene, tooth decay and tooth positioned incorrectly may also increase the risk of periodontal disease [9].

The management and therapy of periodontal diseases may be diverse and is usually adjusted depending on particularities of each case/patient (**Figure 1**). Since periodontal disease occurs when a bacterial biofilm (dental plaque) adheres to the boundary between the teeth and gingiva, causing chronic inflammation and progressively destroying the periodontal tissue that supports the teeth, the periodontal treatment involves scaling and root planning, which mechanically removes the causative bacteria biofilm together with the necrotic cementum from the surface of the tooth root. Appropriate application of this therapy eliminates periodontal tissue inflammation and stops the process of destruction of the same tissue. However, removing the cause of the disease does not regenerate the lost periodontal tissue to its original state [10].

The main approaches considered in the current therapeutic procedures include the following:

(1) *Nonsurgical periodontal therapy* aims in motivating and instructing the patient in adequate self-care, followed by periodical re-evaluation of the oral hygiene status. The primary goal of nonsurgical periodontal therapy is to control microbial periodontal infection by removing bacterial biofilm, calculus and toxins from the involved periodontal root surfaces [11].

A new nonsurgical therapy is the ozone-therapy; the disinfection power of ozone over other antiseptics makes the use of ozone in dentistry a very good alternative and/or an additional disinfectant to standard antiseptics. Due to safety concerns, initially only dissolved ozone in water and ozonated oils were recommended, but a new device used for the gas application

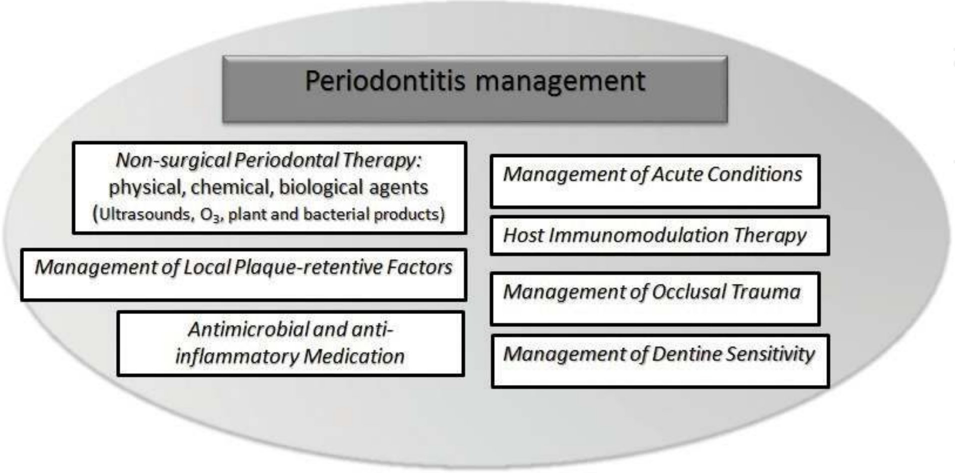


**Figure 1.** Periodontitis management—innovative strategies for reversing the chronic infectious and inflammatory condition.

with a suction feature allows now its safe intra-oral use, with better diffusion even in the dental hard tissues, for its healing and tissue regeneration properties, being indicated in all stages of gingival and periodontal diseases [12].

(2) *Management of local plaque-retentive factors* which refer to mal-positioned teeth, overhanging restorations, crown and bridgework, partial dentures and fixed and removable orthodontic appliances that can increase the risk of periodontal disease and can also prevent successful treatment and resolution of associated pockets. Local irritation and plaque retention caused by untreated carious lesions, subgingival and approximate overhanging crown margins can affect the attachment loss at patients with chronic periodontitis [13].

(3) *Antimicrobial medication* may refer to: full mouth disinfection (consisting in the instrumentation of all periodontal pockets in two steps within 24 h in combination with the adjunctive use of chlorhexidine mouthwash and gel to disinfect any bacterial reservoirs in the oral cavity), local antimicrobials (i.e., disinfectants such as chlorhexidine and locally delivered antibiotics or antiplaque mouthwashes) which have bacteriostatic and bactericidal activity and can inhibit the development of gingivitis, but despite this proved effect, they have a much reduced effect on established plaque and cannot prevent the progression of periodontitis [14], and systemic antibiotics (which are prescribed as an adjunct to root surface instrumentation) have been proposed to act by suppressing the bacterial species responsible for biofilm growth, leading to a less pathogenic oral environment [15].

(4) *Management of acute conditions* should be made as much as possible by local treatment, avoiding the use of systemic antibiotics if there is no significant sign of infection. The main acute conditions that may be associated with periodontal disease refer to periodontitis associated with endodontic lesions (which is a combined perio-endo lesion characterized by clinical attachment loss but also a tooth with a necrotic, or partially necrotic, pulp), periodontal abscess (occasionally occurring in patients with periodontitis, characterized by localized pain and swelling due to nondraining infection of a periodontal pocket), and necrotizing ulcerative gingivitis and periodontitis (characterized by marginal gingival ulceration with loss of the interdental papillae and a gray sloughing on the surface of the ulcers) [9].

(5) *Management of occlusal trauma* have been linked with periodontal disease for many years, but the role of occlusion in the etiology and pathogenesis of inflammatory periodontitis is still not completely understood [16].

(6) *Management of dentine sensitivity* is a condition some patients may experience following root surface instrumentation, especially those with sensitive teeth prior to treatment. Identification and treatment of the causative factors of dentine sensitivity help to prevent the condition from occurring or recurring. There are various treatment modalities available which can be used at home or may be professionally applied, such as toothpastes, mouthwashes, or chewing gums, and they act by either occluding the dentinal tubules or blocking the neural transmission [17].

(7) *Host modulation therapy* aims to modulate the destructive aspects of the host’s immune-inflammatory response to the microbial biofilm by utilizing the anti-inflammatory drugs or oral products (i.e., sub-inhibitory doses of tetracycline). This approach has led to the emergence of a new field of “Perioceutics” which is based on the use of pharmaco-therapeutic

agents including antimicrobial drugs, as well as host modulatory therapy for the management of periodontitis. These host-modulating agents could be successfully used as adjunct components to the balance between periodontal health and disease progression in the direction of a healing response [18].

(8) *Dental prophylaxis* refers to various approaches including plaque elimination by regular tooth brushing, periodical professional removing of mineralized dental plaque or tartar, oral examination and evaluation of periodontal disease progression [19].

## 2. Oral microbiota: host interactions

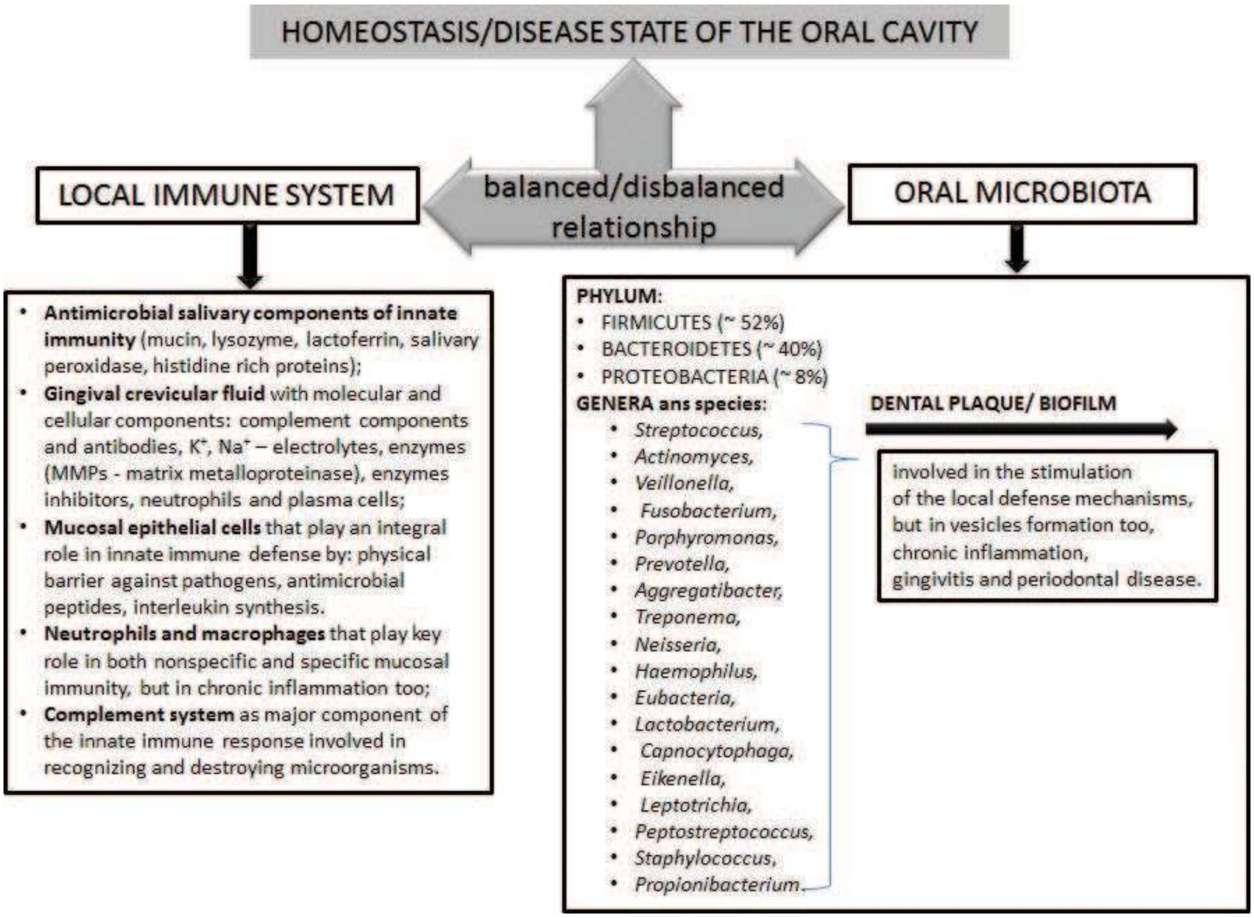
In 2001, Joshua Lederberg introduced the term microbiome signifying “the ecological community of commensal, symbiotic, and pathogenic microorganisms that literally share our body space and have been all but ignored as determinants of health and disease” [20]. These complex communities of microbes and their genes play a fundamental role in controlling the host physiology (metabolism, nutrition, immune system development, regulation of gastrointestinal and cardiovascular systems, etc.) [21] and also support the innate and adaptive host defenses in excluding exogenous (and often pathogenic) microorganisms [22]. The healthy microbiome in any individual patient has relatively lower taxonomic diversity, remaining relatively constant over time, this natural balance being termed “microbial homeostasis,” but its exact composition differs significantly across individuals [23].

The healthy state is highly regulated by the host immune system, and interactions between the microbial community members and with the host maintain a community dominated by “good” microbes, usually gram-positive *Actinobacteria* or streptococci (**Figure 2**) [24].

### 2.1. Host defense mechanisms

Host defenses play an important role in maintaining the homeostasis of the oral cavity. The alliance between the immune system and oral microbiota is responsible for the maintenance of tolerance to microbial antigens, the host monitoring and responding permanently to the colonizing microorganisms. Any changes in this symbiotic relationship induced by antibiotics, diet, and elimination of normal microbiota constitutive species increase the risk for autoimmune and inflammatory disorders [21].

Regarding the prenatal development of cellular components associated with the oral mucosa associated immune system, it was observed that the initial organization of Peyer’s patches can be immunohistologically detected at 11 weeks of gestation [25]. Epithelial cells positive for the secretory component of the sIgA and immunocytes positive for IgM can be detected in salivary gland tissue by 19–20 weeks and continue to predominate during gestation. After birth, immunocytes secreting IgA begin to dominate, but no IgA can be detected in saliva at birth. sIgA was detected in the neonates’ saliva as early as 3 days after birth, and its concentration increased more rapidly during the first 6 months after birth in infants exclusively breast fed [26]. Salivary IgA in young infants has the molecular characteristics of secretory IgA and predominates in saliva. Both IgA subclasses are present in the proportions characteristic of



- **Antimicrobial salivary components of innate immunity** (mucin, lysozyme, lactoferrin, salivary peroxidase, histidine rich proteins);
- **Gingival crevicular fluid** with molecular and cellular components: complement components and antibodies, $K^+$, $Na^+$ – electrolytes, enzymes (MMPs - matrix metalloproteinase), enzymes inhibitors, neutrophils and plasma cells;
- **Mucosal epithelial cells** that play an integral role in innate immune defense by: physical barrier against pathogens, antimicrobial peptides, interleukin synthesis.
- **Neutrophils and macrophages** that play key role in both nonspecific and specific mucosal immunity, but in chronic inflammation too;
- **Complement system** as major component of the innate immune response involved in recognizing and destroying microorganisms.

**PHYLUM:**
- FIRMICUTES (~ 52%)
- BACTEROIDETES (~ 40%)
- PROTEOBACTERIA (~ 8%)

**GENERA ans species:**
- *Streptococcus,*
- *Actinomyces,*
- *Veillonella,*
- *Fusobacterium,*
- *Porphyromonas,*
- *Prevotella,*
- *Aggregatibacter,*
- *Treponema,*
- *Neisseria,*
- *Haemophilus,*
- *Eubacteria,*
- *Lactobacterium,*
- *Capnocytophaga,*
- *Eikenella,*
- *Leptotrichia,*
- *Peptostreptococcus,*
- *Staphylococcus,*
- *Propionibacterium.*

involved in the stimulation of the local defense mechanisms, but in vesicles formation too, chronic inflammation, gingivitis and periodontal disease.

**Figure 2.** The microbiota plays a fundamental role on the induction, training and function of the host immune system, the interactions between mucosal surfaces and microbiota accomplishing a key role in host defense, health and disease.

adult in 1- to 2-month-old infants, although the appearance of IgA2 is delayed in some subjects [27]. The infant apparently can activate mucosal immune responses quite early in life. For example, salivary antibody specific to organisms that originally colonize the oral cavity (e.g., *S. mitis*, *S. salivarius)* can be detected by 1–2 months of age. Most of these antibodies are sIgA, although some IgM antibodies can also be initially detected. Salivary sIgAl and sIgA2 specific to *S. mitis* and *S. salivarius* components increase qualitatively and quantitatively during the first few years of life [28]. Salivary IgA specific to components of streptococci that require hard surfaces for colonization (e.g., *S. sanguis* and *mutans* streptococci) generally appear after tooth eruption [29]. The maternal placental–derived IgG with specificity toward oral microbiota is replaced by the *de novo* synthesis stimulated by the teething process. The collective contributions in the oral cavity of innate and antibody-based immune elements from the saliva, gingival crevicular fluid (and milk if breast feeding) may be considered together with diet, infectious dose, salivary receptors, and tooth integuments, as factors that can determine the outcome of initial colonization events on erupting tooth surfaces [25].

Saliva plays an important role in maintaining the oral homeostasis, through the flushing effects and its antimicrobial constituents like mucin, lysozyme, lactoferrin, salivary peroxidase, and histidine-rich proteins, which are all components of innate immunity [25]. Moreover, local concentrations of these proteins near the mucosal surfaces, periodontal sulcus (gingival crevicular fluid), and oral wounds reinforced by immune and/or inflammatory reactions of the oral mucosa are primarily responsible for innate immunity [30]. *Lysozyme* is a hydrolytic enzyme that cleaves the carbohydrate components of the cell wall peptidoglycan, resulting in cell lysis. This enzyme is active against both gram-negative and gram-positive microorganisms; its targets include *Veilonella* species and *Actinobacillus actinomycetencomitans* [30]. *Lactoferrin* is an iron-binding glycoprotein that links to free iron in the saliva, causing bactericidal or bacteriostatic effects on various microorganisms requiring iron for their survival. Lactoferrin also provides fungicidal, antiviral, anti-inflammatory, and immunomodulatory functions [31–33]. The *lactoperoxidase-thiocyanate system* in saliva has been shown to be bactericidal on some strains of *Lactobacillus* and *Streptococcus* by preventing the accumulation of lysine and glutamic acid, both of which being essential for bacterial growth [34, 35]. The *histatins*, a family of *histidine-rich peptides,* have antimicrobial activity against some strains of *Streptococcus mutans* and inhibit hemagglutination activity of the periodontopathogenic *P. gingivallis.* Also, they neutralize the lipopolysaccharides of gram-negative bacteria and exhibit fungicidal activity against several *Candida* species, *Aspergillus fumigatus*, some strains of *Saccharomyces cerevisiae*, and *Cryptococcus neoformans* [36, 37]. Some defense proteins, like chaperones HSP70/HSPAs (70 kDa heat shock proteins), are also involved in both innate and acquired immunity [30]. In addition, saliva contains abundant CD14 amounts from salivary glands in a soluble form, although LPS-binding protein was below detectable levels, suggesting that saliva CD14 is important for the maintenance of oral health [38].

Gingival crevicular fluid (GCF) is a fluid coming from the junctional epithelium of the gingiva that carries all key molecular (complement components and antibodies, K, Na–electrolytes, enzymes and enzymes inhibitors) and cellular (neutrophils and plasma cells) components of the immune response that are necessary to prevent tissue invasion by subgingival plaque bacteria [39, 40]. Composition of the GCF is the result of the interaction between bacterial biofilm adherent to the tooth surfaces and the cells of the periodontal tissues [41]. The GCF induces permanently changes in microbiota composition, playing an important role in the introduction of immune cells, and being a source of nutrients for resident microorganisms. A number of enzymes can be detected in GCF, including collagenases and elastases which are derived from phagocytic cells and are responsible for the destruction of gingival tissues [42]. Therefore, GCF components might serve as potential diagnostic or prognostic markers for the progression of periodontitis [23, 39]. Although the presence of cytokines was highlighted in GCF, there is no clear evidence of their involvement in the disease. However, interleukin-1 (IL-1) alpha and IL-1 beta are known to increase the binding of PMNs and monocytes to endothelial cells, stimulate the production of prostaglandin E2, release of lysosomal enzymes and bone resorption. On the other hand, interferon (IFN) alpha present in GCF has a protective role in periodontal disease because of its ability to inhibit bone resorption activity of IL-1 beta [43].

Mucosal epithelial cells play an integral role in innate immune defense by sensing signals from the external environment, generating various molecules to affect growth, development, function of other cells and maintaining the balance between health and disease [44]. Mucosal epithelial cells produce antimicrobial peptides that include the β-defensin family, cathelicidin (LL-37), calprotectin, and adrenomedullin. These epithelial antimicrobial peptides are important for wound healing and cell proliferation or exert chemotactic effect to immune cells [45]. It is now recognized that the antimicrobial peptide hBD-2 found in the supra-basal layer of epithelium, stimulates antigen-presenting dendritic cells that signal the adaptive immune system, in addition to its antimicrobial activity [44, 46]. Also, it has been proposed that LL-37, the only antimicrobial peptide from the cathelicidin family, detected in gingival epithelium may be the product of neutrophils migrated through gingival epithelium rather than epithelial cells themselves [47]. LL37 is active against both gram-negative and gram-positive bacteria including established periodontopathogens, like *P. gingivalis* and *A. actinomycetemcomitans* [48].

Calprotectin is constitutively produced by neutrophils, monocytes, macrophages, and epithelial cells, its levels being positively correlated with the severity of periodontitis in GCF [49]. The antimicrobial activity of calprotectin is provided by its ability to bind calcium, zinc, copper, and manganese ions. These ions are essential for usual microbial functioning; thus, calprotectin is a growth inhibitory type of host defense [50].

Oral mucosal cells such as epithelial cells are thought to act as a physical barrier against the invasion of pathogenic organisms, but they have also the ability to produce inflammatory cytokines and express adhesion molecules. Gingival tissue of clinically healthy human also expresses low levels of a wide range of toll-like receptors (TLRs), including TLR1-TLR9 that mediate the response to a broad range of microorganisms [51, 52]. However, oral epithelial cells are refractory to many bacterial components, although they express toll-like receptors/MyD88 and acquire responsiveness after priming with IFN-gamma. When the cells are stimulated with lipopolysaccharides and neutrophil protease (PR3) after IFN-gamma priming, the cells produce interleukin 8 (IL-8), which is critical to Th1 and Th2 responses. PR3 itself is able to activate the cells through G protein-coupled protease-activated receptor-2 on the cell surface.

Also, gingival fibroblasts are well equipped to respond to bacterial components and may contribute to the IL-8 levels observed in clinically healthy tissue [53]. Studies in germ-free mice show that there are low levels of innate immunity mediators present in the periodontal tissue [54, 55], indicating that a basic level of cytokine expression is genetically programmed, without bacterial challenge. Any changes of dental plaque composition modify cytokine expression [54, 56]. As an example, an *in vitro* study is showing that the TLR response can be manipulated by *P. gingivalis* toward two types of lipopolysaccharides: PgLPS1690 (type I) and PgLPS1435/1449 (type II). Type I is a TLR4 agonist, thus activating the immune system, while type II is a TLR4 antagonist inhibiting the immune response to *P. gingivalis* [57]. The expression of these two types of LPS is regulated by the concentration of iron from the hemin found in the GCF [58]. During inflammatory process, *P. gingivalis* type II LPS expression increases which reduces the TLR4 response. It was proposed that this could facilitate survival and multiplication of the entire microbial community [59]. *P. gingivalis* can block gingival epithelial cells IL-8 production *in vitro*, by secreting a serine phosphatase that inhibits the synthesis of IL-8 [60]. This process can delay the recruitment of neutrophils preventing the proper formation of the neutrophil wall, facilitating initial microbial colonization of the periodontium [61]. Other bacteria such as *T. denticola* are also able to manipulate the interleukin response of the host by yet not understood mechanism(s) [62].

### *2.1.1. Neutrophils*

The primary function of both nonspecific and specific mucosal immunity is to protect the teeth, jaws, gingiva, and oral mucosa against infection. In healthy individuals, periodontal tissue contains a wall of neutrophils, between the plaque and the epithelial surface, and cellular infiltrate located in juxtaposition to the colonized tooth surface, closest to the dental plaque biofilm [63]. Expression of mediators such as interleukin 8 (IL-8), intercellular adhesion molecule (ICAM), E-selectin and β defensin molecules 1, 2 and 3 are required to form this neutrophil defense wall [64–66]. E-selectin is required for neutrophil migration from the highly vascularized gingival tissue, IL-8 is a key neutrophil chemo-attractant produced by epithelial cells, and ICAM facilitates adhesion of neutrophils to the tissue allowing formation of this wall [67, 68].

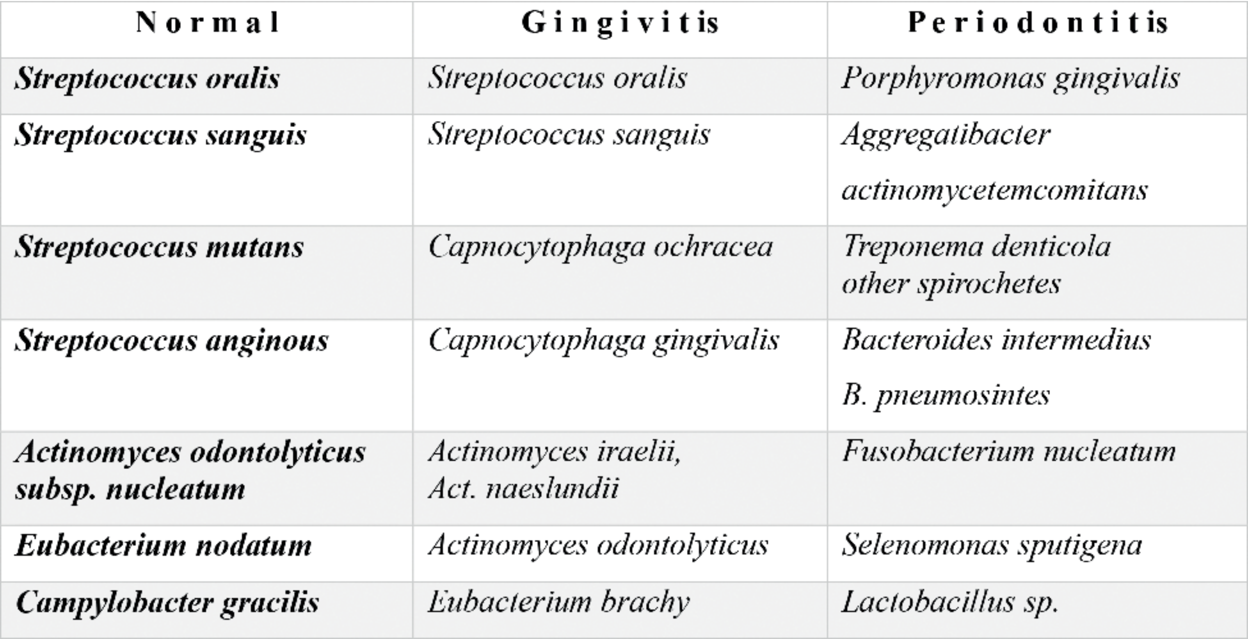
#### *2.1.2. Complement system*

The complement system is a major component of the innate immune response involved in recognizing and destroying microorganisms, with complex roles in homeostasis and disease. Activated complement fragments are abundantly found in the GCF of periodontitis patients, whereas they are absent or present in lower concentrations in healthy individuals [69, 70]. To be a successful pathogen in humans (and any other mammal), a microorganism needs to be able to avoid complement-mediated detection and killing. *In vitro* studies have shown that periodontal bacteria, such as *P. gingivalis*, *T. denticola* and *Prevotella intermedia* could interact with the complement system in complex ways that either inhibit or activate specific complement components [71]. One of the best-studied species from the oral cavity is *P. gingivalis* that produces membrane bound and soluble arginine-specific cysteine proteinases called "gingipains" that can destroy complement factors (C3 and C5) and thus render the bacteria resistant to the bacteriolytical activity of complement system [72, 73].

### **2.2. Oral microbiome dysbiosis and periodontitis**

Recent discussions on the definition of general health have led to the proposal that human health is the ability of the individual to adapt to physiological changes, a condition known as *allostasis* [74]. The *allostasis* in the oral cavity is a complex phenomenon, since the relationship between the oral microbiome and its host is dynamic and any physiological or hormonal changes of the host can affect the balance of the species within these communities [75]. The idea that the accumulation of dental plaque is responsible for oral disease, but without discriminating between the different virulence levels of bacteria, has led to the "Non-Specific Plaque Hypothesis" [76]. The two most common oral diseases, caries and periodontal disease, are highly abundant among the population of industrialized countries, having a major impact on the populations' well-being and healthcare providers [77].

The factors responsible for the transition from periodontal health to either gingivitis or periodontitis are the acquisition of certain species/combinations of species (**Table 1**) and less than optimal host response, which in extreme cases removes the local environment by causing loss of dentition to protect the host from life-threatening bacterial infections [78]. Although the periodontal disease microbiomes are more diverse in terms of community structure, that structure is quite similar across different patients [79]. Gingivitis is associated with an increased microbial load ($10^4$ to $10^6$ organisms) and a corresponding increase in the percentage of gram-negative organisms (15–50%) [80]. An interesting finding is that the microbiota of older subjects, with no prior history of gingivitis, had up to 45% gram-negative species including *Fusobacterium*



| Normal | Gingivitis | Periodontitis |
|---|---|---|
| ***Streptococcus oralis*** | *Streptococcus oralis* | *Porphyromonas gingivalis* |
| ***Streptococcus sanguis*** | *Streptococcus sanguis* | *Aggregatibacter actinomycetemcomitans* |
| ***Streptococcus mutans*** | *Capnocytophaga ochracea* | *Treponema denticola*<br>*other spirochetes* |
| ***Streptococcus anginous*** | *Capnocytophaga gingivalis* | *Bacteroides intermedius*<br>*B. pneumosintes* |
| ***Actinomyces odontolyticus subsp. nucleatum*** | *Actinomyces iraelii,*<br>*Act. naeslundii* | *Fusobacterium nucleatum* |
| ***Eubacterium nodatum*** | *Actinomyces odontolyticus* | *Selenomonas sputigena* |
| ***Campylobacter gracilis*** | *Eubacterium brachy* | *Lactobacillus sp.* |

**Table 1.** The specific composition of subgingival bacterial plaque in gingivitis and periodontitis.

*nucleatum, P. gingivalis, P. intermedia, Campylobacter rectus, Eikenella corrodens, Leptotrichia,* and *Selenomonas* species, demonstrating that an individual is more likely to carry gram-negative bacteria and periodontal pathogens in healthy sites with increasing age [59, 81]. It is likely that the presence of these periodontal pathogens in healthy sites alters the host response, rendering these sites more susceptible to active disease in the future.

The microbiota associated with periodontal disease seems to display a significant enrichment in specific metabolic pathways, compatible with an oxygen poor environment, and the availability of amino acids and lipids as major carbon and nitrogen sources [82]. The unbalanced microbiota is rich in lipid-degradation pathways, as well as other known virulence-related activities, such as lipopolysaccharide (LPS) biosynthesis with local inflammatory effect [83].

The presence of pathogens within this community can lead to the clinical manifestations of periodontal disease, which in turn can lead to additional changes in the community due to the increased availability of nutrients released by the damaged tissue.

Although the bacteria rarely invade the tissues and cause acute infections (e.g., *Prevotella intermedia* invades both epithelial cells and macrophages), they could release substances that penetrate the gum and directly causes the tissue destruction by the enzymes and endotoxins action or indirectly through the induction and maintenance of the chronic inflammatory process, leading to the progressive destruction of collagen in the connective tissue that hold teeth in the gum [84, 85]. The presence of *P. gingivalis* and high colonization *by A. actinomycetemcomitans, T. denticola* and *P. intermedia* plays an important role in severe periodontitis.

The presence of *P. gingivalis* in the dental plaque biofilm results in the inhibition of components of the innate host defense system. This is caused by the lack of IL-8 that normally guides leukocytes to the site of bacterial colonization. In addition, E-selectin that facilitates leukocyte exit from the vasculature into surrounding tissue is absent. The local inhibition of these inflammatory mediators results in the lack of sufficient leukocytes to properly control dental plaque growth, proposed to be one of the major factors in the development of periodontitis. Also, it has been demonstrated that individuals co-infected with *T. denticola* and *P. intermedia* were more likely to have periodontitis than were those infected with a single pathogen [86].

Gram-negative bacteria in particular are known to release large amounts of cell wall material as outer membrane vesicles containing lipopolysaccharide, lipid and protein that are believed to represent a normal mechanism of membrane turnover. In addition, the release of membrane vesicles and cell wall fragments serves to protect bacteria in the biofilm by acting as decoys that bind and activate innate host defense components (i.e., alternative pathways of activation of complement system) and that would otherwise bind to the surface of viable bacteria and kill them; in the same time, the total amount of LPS and the consecutive inflammatory effect are increasing [59].

Lipopolysaccharide has been reported to pass through an intact epithelial cell barrier and concentrate around blood vessels in the *lamina propria*, interacting with nearly all cell types present in the periodontium, inducing a large increase in the numbers of leukocytes, especially neutrophils, in the sulcus or pocket, causing ulcerations [87]. At an early stage, the infiltrate is dominated by B and T (Th1 and Th2) lymphocytes. Subsequently, the lesion becomes dominated by B cells, and less T cells, macrophages and neutrophils, all of which becoming activated. As the disease worsens, periodontal pockets deepen, the components of the extracellular matrix of the gingiva and periodontal ligament are destroyed, and alveolar bone is desorbed [88]. On the other hand, bacterial LPS can subsequently interact with macrophage or dendritic cell receptors, including CD14 and TLRs, to stimulate the production of inflammatory cytokines, especially IL1, and other proinflammatory mediators [89].

The collagen and other components of the perivascular extracellular matrix are destroyed, by the release of lysosomal enzymes by phagocytes and the production of cytokines that stimulate the release of metalloproteinases (including collagenase) by connective tissue cells or cytokines that activate bone resorption [90]. Four distinct pathways may be involved with this destruction: plasminogen dependent, phagocytic, osteoclastic and matrix metalloproteinase (MMP) pathway which is the most prevalent, as revealed by the larger amounts of collagenase and gelatinase (MMP1, MMP2, MMP9, and MMP13) found in the crevicular gingival fluid of patients with periodontitis [91–93]. Cleavage of collagen I by MMP13 seems to be the initial step of the entire bone resorption process [94, 95], and subsequently, denatured collagen fragments are also degraded by gelatinases, MMP2 and MMP9 [96].

Several species, in the subgingival plaque constitution, produce volatile fatty acids (butyrate, propionate, and isobutirat) and peptide N-formyl-methionyl-leucyl-phenylalanine, as sulfide ions, hydrogen sulfide, and methyl mercaptan with cytotoxic effect on endothelial cells and gingival fibroblasts [97, 98].

The bacterial products and epithelial response activate perivascular mast cells to release histamine that activates vascular endothelial cells to release IL-8 within the vessels to assist in localizing neutrophils. B and T lymphocytes are activated by antigens or unspecific mitogens to proliferate and give rise to clones of effector cells; B cells are driven to differentiate into clones of antibody producing plasma cells [99, 100]. Bacterial material released in the periodontium provides thus a major form of communication between dental plaque and the host [78].

Bacteria could activate myeloid cells (e.g., monocytes or neutrophils) to elicit IL-1, and this cytokine then activates a nonmyeloid cell (e.g., fibroblasts, endothelial, or epithelial cells) to secrete additional inflammatory mediators, interleukins 6, 10 and 12, tumor necrosis factor alpha, prostaglandin E2, interferon gamma, and a series of chemotactic substances: monocyte chemoattractant protein, macrophage inflammatory protein, and RANTES (regulated on activation, normal T-cell expressed, and secreted) [88, 101].

After the trend of 1950s and early 1960s, when periodontal treatment was based on the nonspecific plaque hypothesis, criteria for defining periodontal pathogens have been developed.

In periodontal disease, the precise identification of certain organisms (e.g., a particular clone (JP2) of *Aggregatibacter actinomycetemcomitans*) is required to identify the risk factors for localized aggressive periodontitis in young adults. Taking into account the difficulties of periodonto-pathogen cultivation, new techniques have been developed in order to detect bacterial species associated with periodontal disease: PCR-based methods in single or multiplexed approaches, sequencing 16S rRNA gene fragments or a housekeeping gene, next-generation sequencing [102]. Now, the Human Oral Microbiome Database project is undergoing, which aims to catalog all bacterial species found in the oral cavity. However, the molecular techniques are limited to the detection of a selected number of pathogens, so other important disease factors arising from the deregulation of the local host response could be missed [103].

## 3. Mechanisms of dental plaque resistance to antimicrobials and strategies to fight them

Dental plaque displays properties that are typical for biofilms, being structurally and functionally organized polyspecific communities embedded in an extracellular matrix of exopolymers on mucosal and dental surfaces [104], functionally organized and benefiting of increased metabolic efficiency, pathogenic synergism and enhanced virulence, greater resistance to host stress factors and tolerance to all kind of antimicrobials [105].

Resistance to antibiotics (genetic or phenotypical, due to biofilm formation) is the most important cause of nonefficient therapy of biofilm-associated infections from the oral cavity, and it is multifactorial.

One of the main reasons for the antibiotics ineffectiveness against the periodontal pathogen bacteria is that they grow in biofilms, becoming increasingly powerful, aggressive, and difficult to destroy. The antibiotics effective doses and the minimum concentration to eradicate biofilms are very difficult to achieve *in vivo*, especially in the local treatments. Biofilm penetration by biocides or antibiotics is typically strongly hindered. To increase the efficiency of new treatment strategies against bacterial and fungal infections, factors that lead to biofilm growth inhibition, biofilm disruption, or biofilm eradication are being sought. These factors could include antibiotics, e.g., chlorhexidine, triclosan, povidone-iodine active against *P. gingivalis* and *F. nucleatum* biofilms [106]—azithromycin and other macrolides could block quorum sensing mechanism and the alginate polymer formation [107].

The widespread use of antibiotics has evolutionary and ecological consequences, leading to the recruitment of more genes into the *resistome* and *mobilome*, with adverse consequences for human welfare [108], so new approaches are urgently needed to help regain control over infectious diseases, including periodontal disease.

There are theories which support that horizontal gene transfer of resistance determinants can occur in the oral biofilm [109–112]. This strongly suggests that exchange of mobile genetic elements between commensals and pathogenic bacteria can contribute to the emergence of drug resistance in the oral cavity. Oral microbiota exhibits different resistance mechanisms, presumably due to the complex microbial interactions and the genetic fluidity in oral biofilms. The possibility of conjugation among oral bacteria using an erythromycin-resistant (Erm) shuttle plasmid, from *T. denticola* to *S. gordonii,* was revealed [112].

Amoxicillin and penicillin resistance have been described in *Veillonella* sp., *Fusobacterium,* and *Prevotella denticola* isolated from root canals [113, 114]. High levels of penicillin resistance have been demonstrated in the $\alpha$-hemolytic streptococci (*Streptococcus mutans*, *S. salivarius*, *S.oralis,* and *S. mitis*) and represent a cause for concern.

Generally, the $\alpha$-hemolytic streptococci are very highly resistant to cephalosporins; *Enterococcus* sp. isolated from root canal exudates patients with periodontal lesions revealed high-level of cephalosporins resistance [115]. In contrast, Kuriyama et al. found that the genera *Porphyromonas* and *Fusobacterium* showed susceptibility to all cephalosporins, while *Prevotella* species were highly resistant [114].

Mechanisms of metronidazole resistance include mutations in the enzymes responsible for reduction of the antibiotic to its active form, mutations resulting in decreased entry of the antibiotic into the cell and mutations in transporters causing the efflux of the antibiotic. These mechanisms have been demonstrated in different species, e.g., *Lactobacillus* sp., *Gemella morbillorum*, *Actinomyces israelii*, *Clostridium butyricum*, *Eikenella corrodens,* and *A. actinomycetemcomitans* [116]. Four genes, *nimA, nimB, nimC,* and *nimD*, chromosomal or plasmid located, able to confer moderate to high-level metronidazole resistance have been revealed in *Bacteroides* sp. [117].

Mechanisms of tetracycline resistance include efflux proteins, production of ribosome protection proteins, and enzymatic modification of the antibiotic. Tetracycline resistance is encoded by *tet* genes. Antibiotic profiling of $\alpha$-hemolytic streptococci isolated from the oropharynx of healthy Greek children showed a high percent of tetracycline resistant isolates [118], the majority of isolates being represented by *S. mitis*. Okamoto et al. [119] studied the prevalence of *tetQ* gene in genus *Porphyromonas* and *Prevotella* sp. They have been demonstrated that *tet*(M) represented the most common gene, which encodes a ribosomal protection protein, carried on Tn*916*/Tn*1545*-like conjugative transposons in *Streptococcus sp.*, *Granulicatella* sp., *Veillonella sp.* and *Neisseria* sp., from the oral cavity [120].

Resistance to erythromycin is commonly conferred by the acquisition of *erm* gene, antibiotic inactivation by an enzyme encoded by *mph*, and efflux of macrolides by an ATP-binding transporter encoded by *msrA* expressed by *S. aureus* isolates [116]. Low-level macrolide resistance in the oral microbiota may also be associated with the expression of genes from the *mef* family, encoding another efflux pump, and recently have been found on a conjugative transposon Tn*1207.3* in *Streptococcus pyogenes* [121]. There have been described [122] erythromycin-resistant $\alpha$-hemolytic streptococci, such as *S. oralis*, *S. salivarius* and *S. sanguis* in healthy Greek children. *P. intermedia* isolates carried erm(F) alone or tetQ alone, but in other oral anaerobes, macrolide resistance often occurred in conjunction with tetracycline resistance. In *Gemella* sp. and commensal viridans streptococci isolates from oral cavity, macrolide resistance was associated with the *aphA-3* gene [123].

There are studies which support that the resistance to chlorhexidine resistance in *Streptococcus mutans* and S. *sobrinus* isolates from dental plague is plasmid-mediated [124, 125].

Bacteria growing in biofilms often exhibit differing phenotypes compared with their counterpart, respectively planktonic or free cells. There have been described numerous mechanisms for the differences in antibiotic susceptibilities in biofilms relative to planktonic state growth cells. Among these, there have been demonstrated by several studies that oxygen limitation [126], antibiotic penetration into the biofilm [127], the presence of persister cells [128], biofilm-associated cells that grow significantly more slowly than planktonic cells and, as a result, take up antimicrobial agents more slowly [83], and also the maturity of the biofilm might also be important contributors to increased resistance. Results of previous studies have demonstrated also other important mechanisms responsible for resistance to antibiotics [129]: biofilm growth is associated with an increased number of mutations, leading to generation of antibiotic-resistant phenotypes of bacteria, and genes involved in antibiotic resistance are correlated with biofilm phenotype [130]; the production of the exopolysaccharide matrix contributes to an increased cell survival by slowing down antimicrobial diffusion speed; and the differences in metabolic activity among bacteria. It has been revealed that slow-growing and nongrowing bacteria contribute to increased biofilm resistance to antibiotics [131]. The up-regulation of efflux pump proteins and activation of quorum sensing systems reduces and neutralizes incoming antibiotics.

Altered gene expression represents another difference between bacteria grown in biofilms compared with planktonic cells. There are numerous genes that are either positively or negatively regulated by the complex regulatory networks, efflux pumps when the bacteria are growing as a biofilm compared with planktonic cultures [132, 133].

Numerous plants are used in traditional medicine against various diseases. Furthermore, plant extracts have pronounced antimicrobial activities when used at sub-inhibitory concentrations, which are usually very low concentrations with minimal or no effect against host cells. Using sub-inhibitory concentrations of an antimicrobial compound, namely concentrations which do not interfere with bacteria growth, but only with their behavior, leads to reduced risk of developing resistance to that compound. The most recent strategies propose the targeting of communication control, as QS signaling, since quorum sensing is not an essential process, and QS mutants in general have not displayed growth defects, but this signaling controls virulence and biofilms. The quorum sensing mechanism involves the production, release and detection of chemical signaling molecules, which permits communication between microbial cells and gene expression regulation in a cell-density-dependent manner. Granted, interfering with the regulation of virulence factor production developing resistance mechanisms against quorum-inhibiting therapies, may be a difficult proposition for bacteria, which could help promote long-term efficacy of anti-QS therapies [134].

Recent studies revealed that numerous plant-derived compounds and essential oils (EOs) exhibit increased antimicrobial properties, by interfering with QS controlled phenotypes such as adherence, biofilm, formation, motility and pigment production, affecting also antibiotic susceptibility, or revealing microbiostatic properties [135–137]. Not only plant extracts, but also propolis extracts have proved to possess a broad spectrum activity against various gram-positive and gram-negative bacteria: *Staphylococcus* spp., *Streptococcus* spp., *Listeria* spp., *Bacillus* spp., *Enterobacteria* (*Klebsiella pneumoniae*, *Escherichia coli*), *Pseudomonas aeruginosa,* and *Helicobacter pylori* [138, 139]. The antibacterial activity of propolis is mainly correlated with caffeic acids, flavonoids, phenolic esters, and aromatic compounds [140].

Relatively few research works on propolis ability to inhibit biofilm formation have been published. Duarte et al. have shown that propolis inhibits the growth of oral microorganisms and the activity of bacteria-derived glucosyltransferases (GTFs), responsible for glucan synthesis which favors bacterial adhesion and plays an essential role in the development of pathogenic dental plaque [141]. Bulman et al. showed that propolis contains compounds that inhibit signaling mediated by N-acyl-homoserin-lactone in *Pseudomonas aeruginosa PAO1* [142]. Our studies have shown that the 30% Romanian propolis tincture presented antibacterial activity toward *S. aureus, E. coli*, *K. pneumoniae* and *P. aeruginosa*, and antibiofilm activity against *S. aureus*) [139]. Associated with the use of some antibiotics, the efficacy and duration of propolis extract action is more pronounced, and these organisms do not develop antibiotics resistance, as demonstrated for dexpanthenol associated with propolis against *P. aeruginosa* and *S. aureus* strains isolated from infected wounds.

These data confirm that natural extracts have anti-QS, antiseptic, and antivirulence properties and can easily inhibit biofilm formation as well as disrupt the mature biofilm structure. Thus, plant and/or bee extracts in combination with other antimicrobial strategies could provide an effective microbicidal tool for the treatment of various bacterial and yeast infections. However, due to difficulties in cultivating anaerobic periodontal pathogens, there are only few studies concerning the efficiency of vegetal extracts against periodontal pathogenic strains. Chifiriuc et al. (2009) demonstrated that usnic acid selectively inhibited the biofilm development by Gram-positive bacteria and the expression of hemolytic properties of strains isolated from the dental plaque [143].

It has been shown that mouthwash with essential oils (EO) might be a reliable alternative to chlorhexidine (CHX) for controlling gingival inflammation, dental plaque development, bacteremia with anaerobic bacteria in patients with mild-to-moderate gingivitis and oral malodor [144, 145]. Moreover, the diluted EO displayed no detectable detrimental effects on human gingival and periodontal ligament fibroblasts, while diluted CHX reduced both cell migration and long-term survival [146]. The regular long-term use of the EO-based mouth rinse improved the efficacy of a 0.05% cetylpyridinium chloride- and fluoride-containing mouth rinse [144]. Linalool and $\alpha$-terpineol exhibited strong antimicrobial activity against periodontopathic and cariogenic bacteria [147]. *Salvadora persica* root stick extracts and its active component benzyl isothiocyanate are very efficient against oral pathogens involved in periodontal disease as well as against other gram-negative bacteria [148]. The adjunctive use of EO has been shown to promote significant clinical attachment level gain and probing pocket depth reduction in deep residual pockets [149]. A gel containing 10% *Lippiasidoides* (LS) was evaluated and has been shown to reduce plaque, bleeding, and gingival index within the experimental period of 21 days [150].

These scientific data suggest that the antibiofilm compounds should be used in various combinations, in order to develop innovative early combinatory strategies [151] which may potentially strongly support classical treatments and cause an increase of their effectiveness in case of chronic infections, such the periodontal ones.

Other strategies in the improvement of the biological activity used in the management of periodontal diseases are based on the encapsulation of therapeutic drugs in appropriate shuttles. Drug delivery by liposomes with different encapsulated bioactive molecules (such as bacteriocins, enzymes, antiseptics, antibiotics, and vegetal compounds) can assure a controlled delivery of some antimicrobial substances at infection's situs, with a great efficiency in dental caries and every other biofilm-associated infection [152]. Encapsulation technologies, which may shield substances such as nisin from degradation by digestive enzymes and effectively deliver the encapsulated contents at the same time, represent new direction in the field of preventive medicine [153].

## 4. Future perspectives for the therapeutic management of periodontitis

Despite numerous current approaches, periodontitis still remains one of the most common disease, causing moderate-to-very-severe health damage and complications in a high number of individuals. Perspectives for future and advanced therapies aiming for efficient management of periodontal conditions flourished in the last decade, and the recent progress in science and technology made possible a wide range of novel approaches to be successfully applied.

### 4.1. Periodontal tissue regenerative therapy

This approach was formulated in 1993 by Langer, who proposed tissue engineering as a possible technique for regenerating lost periodontal tissues. This rapidly emerging research field represents the interface between materials science and biocompatibility and integrates cells, natural, or synthetic scaffolds, and specific signals to obtain new tissues [154].

Tissue engineering applied for bone and periodontal regeneration combines three key elements to enhance regeneration: use of progenitor cells, design of scaffolds or supporting matrixes, and selection of suitable signaling molecules. Cell sources of progenitor cells may be represented by periodontal ligament-derived cells, periodontal ligament-derived mesenchymal stromal cells, periosteal cells, gingival epithelium and fibroblast cells but also bone marrow–derived mesenchymal stem cells [154].

Scaffolds and supporting matrices are required to offer a three-dimensional (3D) support and assist periodontal tissue regeneration. These structures have important roles, such as: (1) suitable framework, which maintains the shape of the defect; (2) physical support for the healing area, so that there is no collapse of the surrounding tissue into the wound site; (3) 3D substratum for cellular adhesion, migration, proliferation and production of extracellular matrix; (4) selective barrier to restrict cellular migration; and (5) delivery vehicle for growth factors and differentiation molecules [154]. Biomaterials utilized for efficient scaffolds are very diverse, and they may be included in various categories, such as: ceramics (i.e., hydroxyapatite, beta tricalcium phosphate), polymers (i.e., synthetic: polyglycolic acid, polylactic acid and polycaprolactone; natural: collagen fibrin, albumin, hyaluronic acid, cellulose, chitosan, polyhydroxyalkanoates, alginate, agarose, polyamino acids, etc.), and synthetic polyesters (i.e., polyglycolic acid, polylactic acid and polylactic-co-glycolic acid) [155].

In order to increase efficiency, regenerative therapy relies on the incorporation of various bioactive molecules into scaffolding materials, to improve cellular development and tissue healing. The incorporation of specific bioactive molecules within the scaffold is aiming to ensure a sustained and controlled release of bioactive molecules for longer periods of time. These bioactive molecules can be incorporated directly into the scaffolding material or with along with a delivery vector, which ensures its stability and controlled release.

The most utilized bioactive molecules to be integrated in scaffolds designed for periodontal tissue regenerative therapy are as follows: platelet-derived growth factor (potent mediator of periodontal tissue regeneration, currently approved for the treatment of periodontal defects—commercially available as Gem-21 (Osteohealth, Shirley, NY)); fibroblast growth factor (which has a profound effect on periodontal soft tissue and bone healing and also stimulates angiogenesis) [156]; bone morphogenetic proteins (disulfide-linked homodimer that promotes periodontal healing) [157]; insulin-like growth factor (which has mitogenic effects on periodontal ligament fibroblastic cells and can stimulate the synthesis of DNA in periodontal ligament fibroblasts) [158]; transforming growth factor beta (act as bone coupling factor linking bone resorption to bone formation) [159]; and periodontal ligament-derived growth factor (it is a highly specific autocrine chemotactic agent for human periodontal ligament cells, which is 1000-fold more potent than many known growth factors, and has no chemotactic effect on gingival fibroblast or epithelial cells, thereby promising its utility for biological therapeutic regime needed for cell-specific periodontal regeneration) [159].

Recent progress in tissue engineering has allowed the delivery of such molecular factors by various means. Gene therapy and nano-delivery represent the most investigated approaches for the delivery of bio-active molecules, useful for regenerative medicine applied for periodontal disease.

### 4.2. Gene therapy

Gene therapy presents certain advantages when compared to other therapies. Because both cell transplantation and laboratory cell culturing are not needed, gene therapy may be safer and more cost-effective than cell-based therapies [154, 160]. Platelet-derived growth factor and bone morphogenetic proteins are the most utilized for delivery in periodontal regenerative therapy. Plasmid and circular vector-based delivery of platelet-derived growth factor proved safety favorable characteristics for clinical use. Moreover, the expression of platelet-derived growth factor genes was prolonged for up to 10 days in gingival wounds, when administered through this approach. It seems that continuous exposure of cementoblasts to platelet-derived growth factor has inhibitory effect on cementum mineralization, possible via the upregulation of osteopontin and subsequent enhancement of multinucleated giant cells in cementum-engineered scaffolds [161].

The delivery of genes that encode the bone morphogenetic proteins stimulate the formation of periodontal tissue formation. Moreover, the expression of this gene promoted successful regeneration of alveolar bone defects around dental implants [161]. Ribonucleic acid mediated silencing, a novel approach, is based on the principle of RNA interference (RNAi), a novel mechanism of action whereby the expression of certain genes detrimental to the tissue regeneration process is silenced by RNAs. The first siRNA-based therapeutic tested in human clinical trials was the vascular endothelial growth factor (VEGF)–targeted RNA for the treatment of macular degeneration of the retina. Tumor necrosis factor-$\alpha$-targeted siRNA can suppress osteolysis induced by metal particles in a murine calvaria model, opening the way to the application of RNAi in orthopedic and dental implant therapy [162]. The use of RNA-based therapeutics for tissue regeneration is still in its early stages. Nevertheless, RNAi promises to be an effective therapeutic tool and may be successful in periodontal regeneration [154].

## 5. Conclusions

Dental plaque is a model of polyspecific biofilm, very studied mainly due to its accessibility, but also to its implications in dental caries, periodontitis, and periodontal disease—this one being an irreversible affection once launched, very spread in the world. It is of great interest for the field of dentistry and for medicine too, due to its complications, more or less severe, local and at distance too, infectious and noninfectious. A lot of scientific knowledge is accumulated, and therapeutically progresses are done in this field, but the topic still needs improvements and remains a challenge.

Studies have shown that almost all forms of the periodontal disease are consequences of the dental plaque biofilms and of chronic, nonspecific or specific bacterial infections and chronic inflammation too. Along with dental plaque, the occurrence of periodontal diseases is influenced by numerous other factors, such as the virulence and resistance mechanisms of involved microbial species but also host-related factors. Understanding the complex organization of dental plaque biofilms, the interactions between commensal and pathogenic species in this community but also the relation with the host is vital for elucidating the mechanisms of periodontal diseases and drawing novel therapeutic perspectives.

Although traditional preventive and therapeutic approaches relying on adequate hygiene, mechanical removal of the dental plaque, surgery, and antibiotic treatment are still widely utilized, recent strategies propose the utilization of numerous modern techniques to specifically target particular aspects of periodontal disease. Their implementation depends on the extensive knowledge regarding intimate biological parameters of various periodontal conditions and could be more effective in both the prevention and therapy of such diseases.

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