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Microbial Dynamics and Caries: The Role of Antimicrobials

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1. Introduction

The advancement of technology through the application of molecular techniques for identification and analysis of complex bacterial communities have demonstrated the diversity of the oral microbiota and the presence of numerous strains not previously described. Dental plaque is formed by the initial adhesion of pioneer bacterial species to film acquired from enamel, followed by secondary co-aggregation of these bacteria to other microorganisms of different genera and species. This mature dental plaque has some characteristics of multicellular organisms, such as cooperation mechanisms to obtain nutrients, resistance to environmental and communication stresses in order to regulate their growth (Marsh and Martin, 2009).

The understanding of the dental plaque structure as a microbial biofilm sheds light on the clinical relevance of antimicrobials usage (Zanatta et al, 2007). Biofilms have a more tolerant phenotype to antimicrobial agents, stress and host defenses than planktonic cultures, making them difficult to control (Socransky and Haffajee, 2002). This means that the effectiveness of agents used to prevent dental caries, specifically those compounds targeted to combat cariogenic pathogens, should be evaluated in biofilms rather than in traditional liquid cultures (Tenover, 2006). According to Wade (2010), high concentrations of Chlorhexidine (CHX) nearly eliminate all cells, and this is not interesting for microbiota balance in the oral biofilm. Successful antimicrobial agents are able to maintain the oral biofilm at levels compatible with oral health but without disrupting the natural and beneficial properties of the resident oral microflora (Marsh, 2010).

In this chapter, the etiology of dental caries will be briefly introduced focusing on the role of biofilms for initiation and progression of this disease. It will be followed by a thorough review of literature taking into account recent and novel antimicrobial strategies for biofilm control. Recent advances in anti-plaque agents, including those chemoprophylactic, antimicrobial peptides (anti-quorum sensing approach) and probiotics/replacement therapy will be analyzed. Both the discovery of new and effective drugs to control pathogenic

biofilms as well as new delivery systems for oral environment will be the future focus of this research field.

2. Dental biofilm: Dynamics of biofilm formation

General microbial biofilms are defined as communities of microbes associated with any surface (Costerton et al., 1994). In dentistry the surface can be any tooth tissue (enamel, dentin), dental material or any other surface located in the oral cavity. This microbial complex system, also known as 'dental plaque' is organized as bacterial biofilm community that consists of more than 700 different bacterial species (Aas et al., 2005). It is also important to point out that the diverse community of microorganisms found on the tooth surface as a biofilm is embedded in extracellular matrix of polymers (Marsh, 2004).

Bacterial species are thought to play important role in the maintenance of oral health and in the etiology of oral diseases in humans (Socransky et al., 2002). Oral biofilms develop naturally and the resident plaque microflora contributes to the host defenses by preventing colonization by exogenous species (Marsh, 2003). Mechanisms contributing to colonization resistance include more effective competition for nutrients and attachment sites, production of inhibitory factors and creation of unfavorable growth conditions by the resident microflora (Marsh, 2004).

The composition of oral biofilms varies on distinct anatomical surfaces due to the prevailing physical and biological properties of each site (Bowden et al., 1975; Theilade et al., 1982). An important factor involved in oral biofilm formation is the need for specific intermolecular interactions between bacteria and receptors to occur selectively on the enamel surface. In addition to intermolecular interactions, initial attachment of bacteria on a surface is also mediated by nutrient availability, hydrophobicity and hydrophilicity between cell surface and substratum, proteins specificity (Marsh and Bradshaw, 1995).

Between routine oral hygiene procedures, communities re-form on enamel by sequentially adding bacterial constituents in a predictable manner. All bacteria exhibit the ability to adhere to at least one other species of oral bacteria and usually to multiple species. An inherent characteristic of many species of oral bacteria is their ability to recognize and attach to genetically distinct bacterial cells. This phenomenon is termed co-aggregation and has been linked to biofilm formation and maturation of dental plaque (Kolenbrander & Palmer, 2004). Microbial co-aggregation is also thought to be a universal trait of all biofilm bacteria (Rickard et al., 2003) enabling rapid colonization of surfaces and protection from external conditions contributing to survivability, particularly from antimicrobials (Filoche et al., 2004).

The spatial distribution of bacteria is affected by microbial metabolism which produces gradients in biologically significant factors. Gradients develop in key parameters that affect microbial growth (nutrients, pH, O₂, etc.). This will lead to vertical and horizontal stratification of the biofilm plaque and produce a mosaic of micro-environments (Marsh, 2000). These gradients are not linear and such heterogeneity may explain how organisms with apparently contradictory requirements (e.g. in terms of atmosphere, nutrition) are able to co-exist in plaque, and how they are able to influence the activity of antimicrobial agents at different locations within the biofilm (Marsh, 2003).

Biofilm communities are complex and dynamic structures that accumulate through sequential and ordered colonization of multiple oral bacteria (Kolenbrander et al., 2002). The development of a biofilm like dental plaque can be divided arbitrarily into several distinct phases:

Adsorption of host and bacterial molecules to the tooth surface to form of a conditioning film (acquired pellicle). Pellicle forms immediately following eruption or cleaning (Al-Hashimi and Levine, 1989) and directly influences the pattern of initial microbial colonization (Marsh, 2004).

Passive transport of oral bacteria to the pellicle-coated tooth surface. A non-specific reversible phase involving physic-chemical interactions among salivary bacteria and acquire enamel pellicle creates a weak area of net attraction facilitating reversible adhesion. Subsequently, strong, short-range interactions between specific molecules on the bacterial cell surface (adhesins) and complementary receptors in the pellicle can result in irreversible attachment (Lamont & Jenkinson, 2000) and can explain microbial tropisms towards surfaces. Many oral bacteria possess more than one type of adhesion on their cell surface.

Co-aggregation (co-adhesion) of later colonizers to already attached early colonizers. This co-aggregation that also involves specific interbacterial adhesion-receptor interactions (often involving lectins) leads to increased biofilm diversity (Kolenbrander et al., 2000). Co-adhesion may also facilitate the functional organization of dental plaque (Bradshaw et al., 1998).

Multiplication of attached microorganisms to produce confluent growth: Cell division leads to confluent growth and eventually, a three-dimensional spatially and functionally organized mixed-culture biofilm. Dental plaque functions as a true microbial community in which properties are greater than the sum of the component species (Marsh, 2004).

Active bacteria detachment from surfaces: Bacteria can respond to environmental cues and detach from surfaces, enabling cells to colonize elsewhere.

It has been suggested that oral biofilm formation consisted of two process involving separate mechanisms (Gibbons and van Houte, 1973). The first process was associated with adsorption of cells to the pellicle and required specific adhesions on the cell surface. The second step involved a build-up of cells biding to each other in a process termed co-adhesion.

Bacterial accretion through co-adhesion drives the temporal development of plaque biofilms that is characterized by bacterial successions and occurs over a time frame of weeks. The early biofilm consisted of pioneer organisms deposition followed by multiplication in morphologically distinct palisading columns of cocci (Rosan and Lamont, 2000). Pioneer species are predominantly streptococci (*S. sanguis*, *S. oralis* and *S. mitis*) (Marsh and Bradshaw, 1995). Although the oral streptococci initially predominate in plaque and can constitute up to 80% of early plaque, another significant colonizing species is *Actinomyces naeslundii*, and some haemophili (Rosan and Lamont, 2000).

Single cells of mainly Gram-positive coccoid cells can be seen by microscopy on pellicle-coated surfaces, together with a few rod-shaped organisms, after few hours (2-4h) of plaque formation (Marsh and Bradshaw, 1995). The attached cells then divide rapidly to form microcolonies in the first instance, which coalesce to form a confluent film of varying thickness (Nyvad and Fejerskov, 1989).

After 1-2 days, Gram-positive rods and filaments can be observed extending outwards from microcolonies of mainly coccoid cells. After several days of development, morphological and cultural microflora diversity increases. The biofilm depth increases and its structure becomes more varied. If plaque is left to develop undisturbed on exposed enamel surfaces for 2-3 weeks, a climax community will establish and bacterial composition will become relatively constant overtime (Marsh and Bradshaw, 1995).

A very important key point on biofilm formation is the synthesis of extracellular polyssacharides from sucrose by adherent bacteria (Figure 1). These insoluble molecules are considered very important contributors in the structural integrity and pathogenic properties of biofilms.

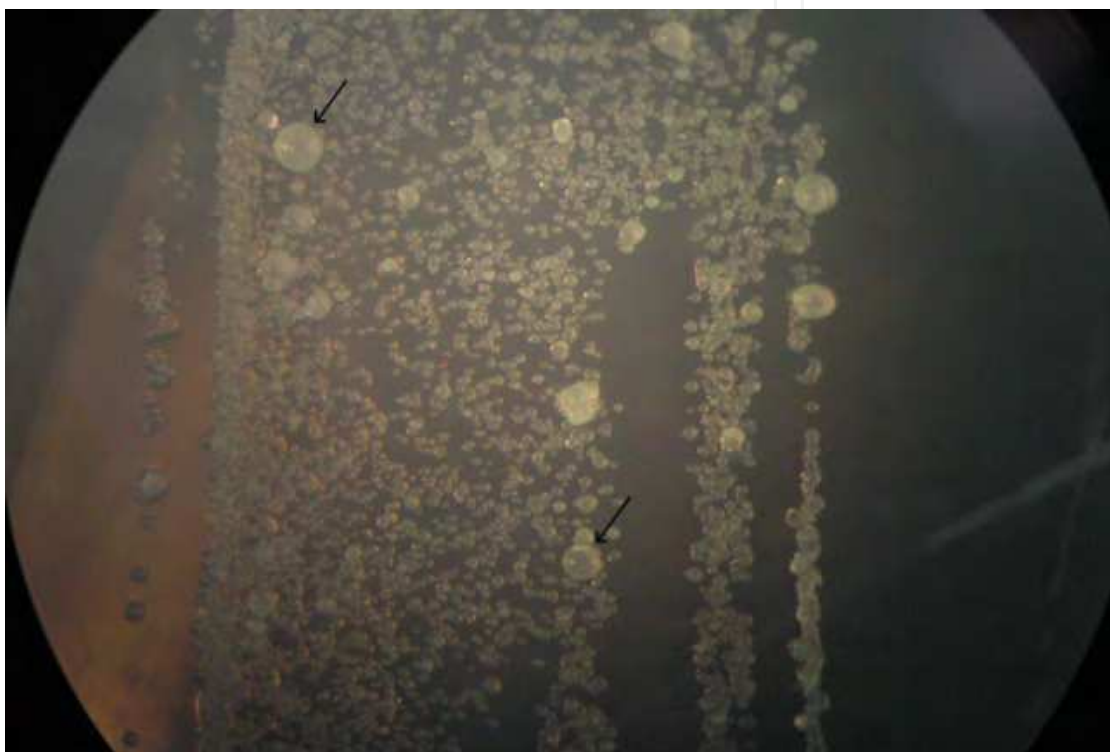


Fig. 1. Production of extracellular polymers of *S. mutans* UA159 under planktonic form of growth with sucrose enriched medium. Note arrows that indicate the presence of polymers. (40x)

Confocal laser scanning microscopy techniques has demonstrated that “dental plaque” has a similar architecture to biofilms from others sites. Dental plaque has open architecture, with channels traversing from the biofilm surface through to the enamel (Wood et al., 2000; Zaura-Arite et al., 2001). This structure will have important implications for penetration and distribution of molecules within plaque.

One of the most notable features of clinical relevance with respect to phenotype of micro-organisms growing on a surface is the increased resistance of biofilms to antimicrobial agents (Mah and O'Toole, 2001). Bacteria growing as a biofilm frequently express phenotypes that are different from those of planktonic bacteria and one possible consequence can be a reduced sensitivity to inhibitors. Most pre-clinical trials for testing oral antibacterial products used planktonic techniques where cells grow freely without any effect

of biofilm structure. Therefore, most products that showed good *in vitro* results did not show similar effects under clinical evaluations (Guggenheim et al., 2004; Leibovitz et al., 2003; Marsh, 2004). The observation that genetic expressions of *S. mutans* are not the same for planctonic and biofilm forms of organization supports that most antimicrobials need biofilm techniques for reproducing more closely to the oral environment *in vivo* (Marsh, 2004).

A greater understanding of the significance of oral biofilms as a complex bacterial structure will have the potential to impact significantly on clinical practice (Marsh, 2004). Biofilms in nature are often difficult to investigate and experimental conditions are not completely defined. Therefore, a number of different laboratory-based experimental biofilm model systems have been developed (Palmer, 1999). These systems allow studies on biofilms under defined conditions. Such systems are necessary in order to perform well-controlled reproducible experiments (Tolker-Nielsen et al., 2000). Various multispecies models of biofilm testing procedures have been described and applied to problems of clinical relevance, most notably biofilm permeability and chemical control. These systems usually consist either of flow cells (Christersson et al., 1987; Larsen and Fiehn, 1995) or chemostats modified to allow for insertion and removal of colonizable surfaces (Bowden, 1999; Bradshaw et al., 1996; Herles et al., 1994), and these devices have contributed to our understanding of microbial adhesion and biofilm formation. In spite of the great development on oral biofilms studies, there is still a room for improvement. Thus, new insights will be presented in the near future on this topic.

3. Communication microbial biofilms: *Quorum sensing* mechanisms

In the dental biofilm bacteria do not exist as independent entities, but as a coordinated and metabolically integrated microbial community (Marsh & Bowden, 2000). This interaction provides enormous benefits to the participating organizations compared to the same bacteria grown in planctonic form, including: a wider range of habitat for growth, increased diversity, increased metabolic efficiency and greater resistance to environment, antimicrobial agents and host defenses (Shapiro, 1998; Marsh & Bowden, 2000).

The heterogeneity and high bacteria density within biofilms promote genotypic and phenotypic changes through releasing of self-inducers in the environment, leading to modification of gene expression (virulence genes of exoenzymes, exopolysaccharides) and at the same time, the acquisition of a important competitive advantage for survival and perpetuation in natural environments, highly competitive (eg, oral cavity, intestine), where hundreds of species coexist (Shei & Petersen, 2004). These virulence factors mediated by self-inductors were called "quorum sensing" (Fuqua et al., 1994).

The *quorum sensing* (QS) is a communication process among microorganisms mediated by population density. The main QS' regulation mechanism is given by production of auto-inducers released into the external environment, where they accumulate, and the interaction with its receptor, which may be intracellular or present in cell surface (Nealson et al, 1970; Hens et al., 2007). When its concentration reaches a certain threshold, it promotes the activation or repression of several genes causing cells to exhibit new phenotypes (Redfield, 2002).

The first indication that bacteria communicate through chemical signals came from studies of Nealson et al. (1970). They studied the bioluminescence regulation in the marine bacterium *Vibrio fischeri*, which has a symbiotic relationship with marine animals such as squid. In this regard, the host uses the light produced by the bacterium, to attract preys and partners or ward off predators, while the *V. fischeri* obtains necessary nutrients from its host (Nealson et al., 1970).

The luminescence is observed only when bacteria colonize the host's organs and by increasing the number of bacteria in the medium they are able to perceive cell density by detecting the auto-inducer concentration. Upon reaching a threshold concentration of self-induction, it is enough to trigger the process of gene transcription (Swem et al., 2009).

The self-inducers involved in this process may be of different chemical nature, in gram-negative organisms the signaling molecules are derived from N-acyl homoserine lactone (AHL) and its regulation occurs through homologous proteins LuxR and LuxI. The first protein acts as an enzyme (AHL synthetase) and second, when connecting to the AHL, forms the AHL-LuxR the complex, which is responsible for the activation and expression of numerous genes. In gram-positive, self-inducers usually correspond to small peptides (hepta and octapeptides). These peptides are usually secreted by carriers bound to ATP (ABC). Some interact with membrane-bound kinases sensors carrying a flag through the membrane; others are transported into the cell by oligopeptide permeases, which then interact with intracellular receptors (Swem et al., 2009; Rock Road, et al., 2010).

In QS systems via AHLs, the variation in the acyl chain (chain length, degree of oxidation and saturation) may confer some specificity to these communication systems. Thus, there seems to be some cross-talk among bacteria belonging to different genera. Part of this cross-talk may represent a way by which bacteria acquire information about the total population, allowing a response to competitors or prospective members (Williams, 2007). *E. coli* does not synthesize AHLs but express a homologous biosensor LUXR (SDIA). It is speculated that this system allows *E. coli* detecting communication signals of other gram-negative and exploiting such information for its own benefit (Ahmer, 2004).

The gram-negative bacterium, *Streptococcus mutans*, a major pathogen of dental caries, performs the quorum-sensing by releasing mediator peptides of gene expression. The signaling system involves at least six gene products encoded *comCDE*, *comAB* and *comX* (Cvitkovitch et al., 2003). The OMCC gene encodes a precursor peptide, which when cleaved and exported release a signal peptide, 21 amino acid or stimulating competence peptide (CSP). Through the quorum-sensing, it was found that the competence-stimulating peptide (CSP) was necessary for proper formation of *S. mutans* biofilm in addition to its virulence characteristics (Li et al., 2001).

The quorum sensing systems control a variety of microbial processes such as sporulation, virulence, biofilm formation, conjugation and production of extracellular enzymes (Miller & Bassler, 2001). Bacteria use QS to coordinate gene expression within species. Moreover, the same detection signals are used to inhibit or activate transcription programs between competing bacteria strains and other existing species in the same microenvironment (Bassler, 2002). Communication can still cross the borders of the kingdom, as QS effector molecules that can alter the eukaryotic transcription programs, found in epithelial cells and immune effector cells (Williams, 2007; Shin et al., 2005).

The discovery that *S. mutans* performs quorum-sensing of the system ideal in growing biofilms led us to investigate other features of this system in biofilm formation and biofilm physiology. The strategy for control of microorganisms by interfering in QS systems presents an important alternative for control of oral biofilms.

4. Antimicrobials: Mechanisms of action

Antimicrobials can be bactericidal (kill the microorganism directly) or bacteriostatic (prevent the microbe growth). In the case of bacteriostatic drugs, host defenses such as phagocytosis and antibody production usually destroy the microorganism. With the suspension of the second type of drug, bacteria can grow back. For bacteriostatic and bactericidal actions are apparent it is necessary to determine the MIC (Minimum Inhibitory Concentration) and MBC (minimum bactericidal concentration). As the therapeutic activity of antibiotics depends, among other factors, on their concentrations in body fluids, MICs and CBMs are essential determinations, since the establishment of the antibiotic regimen depends on them. The MIC and MBC are estimated in vitro, but used to determine bacteriostatic and bactericidal concentrations of antibiotics in body fluids (Maillard, 2002).

In Biofilms, MIC and MBC of antimicrobial agents usually must be greater than those required for plancttonic cells, due to its greater resistance to these drugs. In addition, optimal antimicrobials indicated for diseases that have bacteria organized in biofilms as etiological agent, must have good distribution in these structures. The main mechanisms of action of antimicrobials include: inhibition of cell wall synthesis, inhibition of protein synthesis, plasma membrane damage, inhibition of the synthesis of nucleic acids and inhibition of the synthesis of essential metabolites (Maillard, 2002).

Cell Wall Inhibition. The bacterium's cell wall consists of a network of macromolecules called peptidoglycan, which is found exclusively in bacteria's cell wall. Penicillin and other antibiotics prevent complete synthesis of peptidoglycan, consequently, the cell wall becomes fragile and cell undergoes lysis. As penicillin targets the synthesis process, only cells in active growth will be affected by this antibiotic. And as human cells do not have peptidoglycan, penicillin has low cytotoxicity to the host cell (Broadley et al. 1995).

Inhibition of Protein Synthesis. Protein synthesis is a characteristic common to all cells, both prokaryotes and eukaryotes, not presenting therefore a suitable target for selective toxicity. Eukaryotic cells have 80S ribosomes and prokaryotic cells have 70S ribosomes. The difference in the ribosome structure is responsible for selective toxicity to antibiotics that affect protein synthesis. However, the mitochondria (important cytoplasmic organelles) also has the 70S ribosomal unit similar to bacteria units. Antibiotics that act on the 70S ribosome may therefore have adverse effects on host cells. Among the antibiotics that interfere are the clorofenicol, erythromycin, streptomycin, and tetracycline (Nakamura & Tamaoki, 1968).

Damage to the plasma membrane. Certain antibiotics, especially polypeptide antibiotics, promote changes in the permeability of plasma membrane. These changes result in the loss of major metabolites of the microbial cell. For example, polymyxin B disrupts the plasma membrane by binding to membrane phospholipids (Lambert & Hammond, 1973). Likewise, planktonic cells, when exposed to higher concentrations of the chlorhexidine (CHX), suffer membrane rupture (Figure 2). This observation can be explained by the fact that CHX,

which is positively charged, binds tightly to negatively charged bacteria membrane, causing its disruption (Gilbert & Moore, 2005).

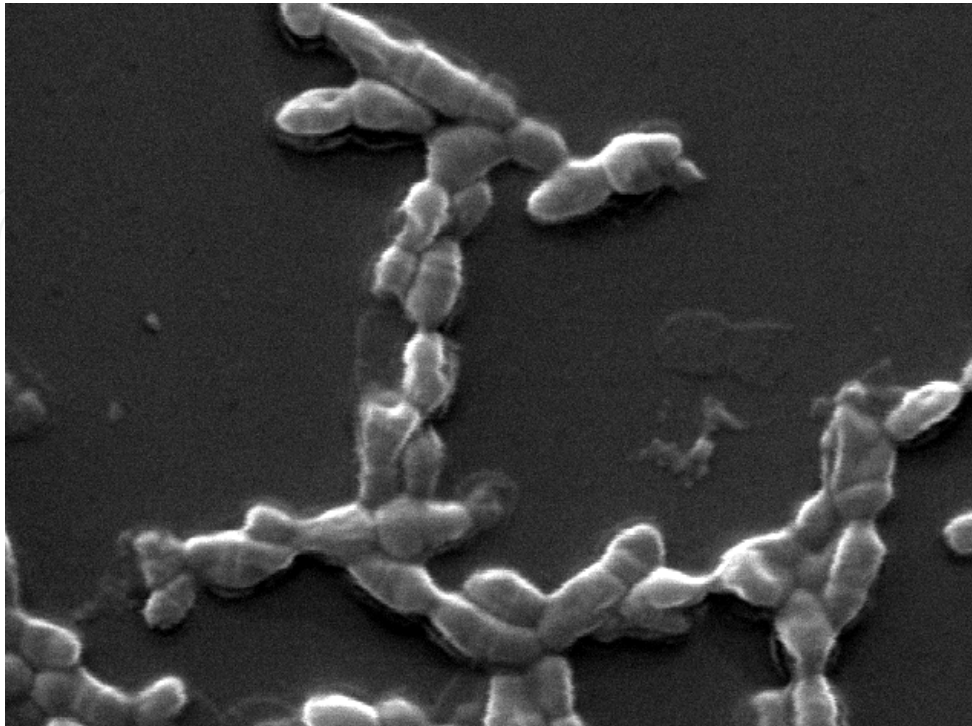


Fig. 2. Scanning electron micrograph of planktonic *S. mutans* UA159 cells exposed to chlorhexidine at 4.5 µg/ml. After 6h of CHX incubation, several wilted cells with spilled intracellular material could be observed (15 kV and 13.000x of magnification).

Inhibition of nucleic acids synthesis. Some antibiotics interfere with the processes of DNA transcription and replication of microorganisms. Some drugs with this mode of action have limited use due to interference with DNA and RNA of mammals. Others, such as rifampin and quinolones, are more widely used in chemotherapy by having a higher degree of selective toxicity (Silver, 1967).

Inhibition of Synthesis of Essential Metabolites. The enzymatic activity of a specific microorganism can be competitively inhibited by a substance (antimetabolites) that closely resembles enzyme's normal substrate (Russell and Hugo, 1994).

5. Recent advances in anti-plaque agents: Chemoprophylactic agents, antimicrobial peptides, anti-quorum sensing approach and probiotics/replacement therapy

Control of oral biofilms is essential for maintaining oral health and preventing dental caries, gingivitis and periodontitis. However, oral biofilms are not easily controlled by mechanical means and represent difficult targets for chemical control (Socransky, 2002). With the exception of chlorhexidine and fluoride, few of the existing oral prophylactic agents have significant effects (Petersen & Scheie, 1998; Wu & Savitt, 2002; Scheie, 2003). A likely explanation for this low efficiency is due to the fact that microorganisms organized in biofilms possess characteristics that differentiate them from planktonic cells, such as higher

resistance to several antimicrobial agents; most studies so far use study models with planktonic cells, not reproducing the reality of the oral cavity. In addition, antimicrobials for oral use must have adequate diffusion in biofilms to be effective (Marsh, 2005).

Thus, many of these studies need to be revalidated, taking into account the oral environment. Recent approaches to the study of microbial gene expression and regulation in non-oral microorganisms have elucidated systems for transduction of stimuli in biofilms, such as two-component systems and quorum sensing (two-component and quorum sensing systems) that allow the coordinated gene expression in these structures. These studies based on understanding the regulation and expression in microbial biofilms can potentially benefit the development of new strategies for prevention and treatment of diseases caused by oral biofilms. Thus, the intervention should be directed at targets such as surface adhesion, colonization, co-adhesion, metabolism, growth, adaptation, maturation, climax community and detachment, and strategies must be based on surface modification, immunization, replacement therapy, interference with two-component systems and quorum sensing (Scheie, 2004).

These new drugs must be highly specific, have little ability to induce resistance in microorganisms and produce minimal effects on vital functions of human cells. In therapeutic approaches, the main target should be the mature and established biofilm. In this case, genes and proteins essential for viability of microorganisms represent the traditional targets for designing these antimicrobial drugs. Among these potential agents are included bacteriophages, inhibitors of the biosynthesis of fatty acids and antimicrobial peptides (Hancock, 1999, Payne et al. 2001; Sulakvelidze & Morris, 2001). In prophylactic approaches, the main targets are the pathogenic microorganisms directly involved in the formation of mono or multi-species biofilms. Promising targets for this purpose would be the two-component systems and quorum sensing, whose inference could be used to ensure the ecological balance in the biofilm, allowing the maintenance of health-related microbiota (Marsh, 2010). This approach would have a selective toxicity, since these systems are present in most microorganisms, but not in mammalian cells, which use other mechanisms of signal transduction.

Another important strategy is the modification of tooth surface or, more precisely, the film acquired from the enamel to prevent bacterial colonization and thus biofilm formation. The film acquired from enamel has binding sites for oral bacteria through specific and nonspecific binding mechanisms. An *in vitro* study showed that the combination of alkylphosphate and a nonionic surfactant changes the characteristics of tooth surface, making it less attractive for microorganisms. However, the clinical efficacy of these agents has been low, probably due to difficulties in obtaining the active components of these agents (Olsson, 1998).

Some properties of topical antimicrobial agents for oral use are essential to their success as high substantivity in the oral sites of biological action, low acute and chronic toxicity, and low permeability, being overall associated with their mechanism of action. Clinical activity of the antimicrobial agent depends on the drug formulation that must have a quick and efficient release vehicle. The supragingival plaque, film acquired from enamel and saliva may be primary sites of action for these agents, but the detailed understanding of these interactions is limited. These antimicrobials are retained by electrostatic bonds to carboxylic

acids and phosphate and sulfate residues of proteins and glycoproteins in the oral mucosa, film acquired from enamel and plaque. The non-ionic antibacterials are retained by adsorption to lipophilic regions in these receptor sites. The ability of these antiplaque agents have to keep an optimal concentration in saliva over a long period, in addition to remaining in the bioactive form at the action sites, such as the teeth surfaces is extremely important and influence in the clinical effectiveness of these agents (Cummins & Creeth, 1992).

The analysis of retention characteristics and antimicrobial properties of clinically proven antiplaque agents suggest that they act multifunctionally and at multiple sites. Thus, they reduce growth and metabolism of bacteria in plaque, saliva and tooth surface, but also reduce the adhesion of potential settlers. Two generic routes to increase the antiplaque activity of these agents have received attention. Firstly the use of a combination of antimicrobial agents with similar but complementary activities uses only one route and mode of action. A second potential route is the use of a polymer that serves as auxiliary retention of only one antimicrobial used (Cummins, 1991b).

The total oral retention, salivary profile and agent concentrations on the plaque, film acquired from enamel and oral mucosa are not only indicators of biological activity in vivo, but they serve as potential indicators for this activity. This means that the increased release of a specific agent in vivo is not predictive of its increased clinical efficacy (Cummins, 1991b). The increased activity on the site or sites of biological action combined with agent's residence time in the oral cavity are the best predictors of agent's clinical activity (Creeth & Cummins, 1992).

Replacement therapy has been suggested as a strategy for replacement of pathogenic microorganisms modified to become less virulent. Some requirements for this type of approach are important, such as: the replaced organism must not cause disease by itself; it must persistently colonize and must possess a high degree of genetic stability. DNA technology has enabled to produce potential candidates for replacement therapy in the prevention of dental caries. Among these, there is the super-colonizing strain of *S. mutans*. This strain produces mutacin, which allows it to replace the wild-type strain efficiently. It lacks the enzyme lactate dehydrogenase and therefore is unable to produce lactate (Hillman et al., 2000). Other ureolytic recombinant strains have been constructed and are capable of hydrolyzing urea to ammonia, thereby offsetting the environment acidification (Clancy et al., 2000).

A possible future approach would be to use genetically modified microorganisms for releasing molecules that could interfere with pathways such as signal transduction of two-component and quorum sensing. However, it is important to emphasize that there is the possibility of a genetically modified strain subsequently undergoes transformation in oral biofilms and then becomes a pathogenic opportunistic strain (Scheie, 2004).

Immunization against oral diseases as dental caries and periodontal disease has been extensively studied in recent decades (Koga et al. 2002; Smith, 2002). The goal would be inhibiting or reducing the virulence of some microbial etiological agents. Several molecules involved in various stages of the pathogenesis of caries and periodontal disease could be susceptible to immune intervention and serve as targets for production of vaccines. Thus, it would be possible to eliminate microorganisms of the oral cavity with antibodies able to block adhesins or receptors involved in adhesion, or metabolically modify important

functions or virulence. Efforts are being made for manufacturing active and passive vaccines, especially for tooth decay. In active immunization, an attenuated antigen induces a protective immune response when administered. In passive immunization, the ready antibody is administered (Sheie, 2004).

Studies on animals and humans using approaches with active and passive immunization have been successful, especially in passive immunization where there is impediment to recolonization of microorganisms related to dental caries (Koga et al. 2002; Smith, 2002) and also in periodontal disease (Booth et al., 1996). The vehicles for passive immunization, such as milk from immunized cows (Shimazaki et al., 2001) and transgenic plants (Ma et al., 1998), have been tested with promising results. Similarly, it was shown that recombinant chimeric microbial vectors that are non-virulent, but express antigens of *S. mutans* (Huang et al., 2001, Taubman et al., 2001) or *P. gingivalis* (Sharma et al., 2001) promoted protection against tooth decay and loss of alveolar bone in experimental animals.

One of the issues that still need to be solved is about which immune system should be stimulated, if the systemic immune system or that associated with mucosal. In the case of an anti-caries vaccine, it would be more interesting the oral administration and based on induction of the immune system associated with mucosa. A vaccine against periodontal disease should probably involve the systemic immune system and that associated with mucosa. A major problem is that approaches to immunization are usually directed against epitopes of isolate bacteria; however, both tooth decay and periodontal disease are diseases whose etiologic agent consists of a multispecies microbiota (Marsh, 1994). Moreover, since microorganisms have the ability to form biofilms and adapt to this environment, this can lead to changes in antigenicity which could affect the durability of protection induced by immunization.

An alternative approach are a new class of antibiotics called of antimicrobial peptides (AMP) that can be used against these microorganisms (White et al., 1995, Yount & Yeaman, 2004; Hancock & Sahl, 2006; Gardy et al., 2009); however, a poor understanding of the fundamental principles of the action mechanisms and structure-activity relationship of these drugs (Shai, 2002; Bechinger, 2009) has reduced the development of MPAs that can be used clinically.

The potential advantages of using antimicrobial peptides as antimicrobial drugs are significant (Hamill et al., 2008, Easton et al., 2009). They have a broad spectrum of activity against many strains of Gram positive and negative bacteria, including strains resistant to other drugs, and are also active against some fungi. Moreover, their interactions with bacterial components do not involve binding sites to specific proteins and thus do not induce resistance. The AMP bioavailability is reduced because they cannot be taken orally; however, topical applications and injections are available. Recently, AMP has been tested in clinical trials for various applications in oral candidiasis (Demegen Pharmaceuticals, 2010), infections associated with catheters (Melo et al., 2006) and infections in implant surfaces (Kazemzadeh-Narbat et al., 2010). These clinically tested AMPs are derived from natural peptides, this fact being responsible for its biggest drawback, which is the high production cost compared to other chemical antibiotics. For this reason, there is a critical need for development of new AMPs, powerful, small and with simple composition. The potential for the rational design of these drugs is often limited due to little knowledge about the details of its mechanism of action.

Since tooth decay is an infection, it would be logical to treat the disease with antibiotics or antimicrobials, such as antimicrobial peptides. However, most of these agents are not selective, have broad spectrum of action on the microorganisms such as chlorhexidine, iodopovidine, fluoride, penicillin or other antimicrobial/antibiotics. Importantly, the agents described above does not sterilize the oral cavity, since it is exposed to the external environment where there are many microbes, it is not a sterile space. Thus, the use of broad-spectrum agents for treating dental caries can suppress the infection, but will never eliminate it entirely (Luoma et al., 1978). In this context, due to the limitations of traditional strategies in the management of dental caries, a "probiotic" approach of the disease is necessary. The term "probiotic" used here means that mechanisms are used to selectively remove only the pathogen responsible for disease in an attempt to keep the oral ecosystem intact. Most efforts in this sense are derived from studies that have attempted to genetically modify strains of *Streptococcus mutans*, turning them into strains that in addition to not producing acids, still competing for the same ecological niche that wild strain of *S. mutans* (Hillman, 2002).

In theory and experimentally in laboratory animals, when this substitute organism is introduced, it completely shifts the wild *S. mutans* causing the disease. This action stops the decay process and also prevents the re-emergence of disease-causing organisms, eliminating the possibility of re-infection, since the "normal microbiota is complete." Another way to remove pathogens is developing specific antimicrobials for certain targets (Eckert et al., 2006). The basic principle is developing a cheap molecule that targets only the organism of interest, in this case *S. mutans*, *S. sobrinus*, or other pathogens.

In the case of the oral cavity and tooth decay, this system is attractive from the perspective of eliminating all pathogens, thus preventing the re-growth of the original infection. There are also laboratory and clinical evidence demonstrating that when the biofilm's bacterial ecosystem is free of *S. mutans*, this bacterium finds it difficult to be reintroduced due to competitive inhibition with other microorganisms (Keene & Shklair, 1974; Shi, 2005). A criticism to probiotic approaches is that they target only one of the pathogens involved with the disease, being not directed at other pathogens that may be involved with the beginning of the process, as the case of dental caries.

6. The role of antimicrobials in the future

A better understanding of bacterial communities found in biofilms, such as its diversity and interactions among cells, provides opportunities for new methods to control biofilm formation (Wade, 2010). It has been shown that blocking communication mechanisms between cells in biofilms (quorum-sensing) can partially restore their susceptibility to antimicrobial agents (Bjarnsholt et al., 2005). Other benefits may include reduction of pathogenic microorganisms due to reduction in the virulence mechanism in the microorganism of interest. In the particular case of dental caries, blocking or reducing the activity of glycosyltransferase in *S. mutans* would be interesting, since these enzymes are implicated in the ability of this cariogenic bacterium.

In addition, probiotic approaches for oral use are being developed: such as the development of *Lactobacillus paracasei* strains which maintain its co-aggregation activity with *S. mutans* even when dead (Lang et al., 2010), or *Lactobacillus reuteri* strains that are able to reduce the

number of *S. mutans* in the mouth (Caglar et al., 2008) in order to decrease the incidence of tooth decay. Other bacterium normally found in the mouth, and important in this sense, is *Streptococcus salivarius*, which produces a bacteriocin that inhibits anaerobic Gram-negative bacteria and that in vivo was shown also to reduce the level of halitosis (Burton et al., 2006).

In conclusion, microbiota analysis methods independent of culture have allowed to understanding the diversity of the oral microbiota. So far, most studies have focused on the microbiota composition in the disease, but a better understanding of this microflora in health is required and also as probiotic organisms are capable of restoring and maintaining health in such environment. Thus, future studies are still needed, especially for analyzing the interactions between species and how to use this knowledge to develop new products for prevention and treatment of oral diseases.

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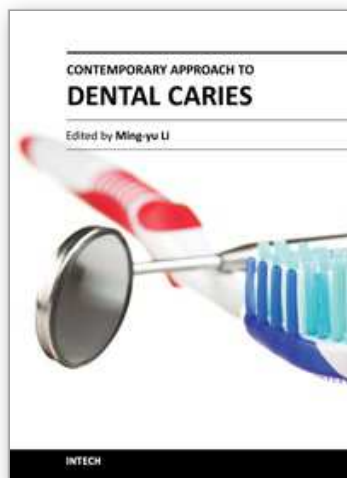
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