academicJournals

Vol. 6(5), pp. 51-57, August 2014
DOI: 10.5897/JDOH2014.0127
Article Number: 84910BB46956
ISSN 2006-9871

http://www.academicjournals.org/JDOH

Journal of Dentistry and Oral Hygiene

Review

Oral bacterial interactions in periodontal health and disease

Ismail A. Darout

Department of Preventive Dental Sciences, College of Dentistry, Jazan University, Kingdom of Saudi Arabia.

Received 2 June, 2014; Accepted 26 June, 2014

Periodontal disease and dental caries are infectious diseases resulting from the interactions of oral bacteria residing dental plaque and the host. The indigenous bacteria residing dental plaque are thought to be a relatively stable community of high species diversity, which may vary from site to site throughout the mouth. When this stability is disturbed, due to many host-specific and environmental factors, in addition to oral hygiene and dietary habits which the subjects can regulate, other less benign bacteria may colonize the oral cavity and the bacteria that are normally present in very low number may increase to cause oral diseases. The aim of this review paper is to highlight the oral microbial ecosystems in oral health and disease and to investigate the ecological changes that shift the indigenous bacteria residing dental plaque to be increased in number and cause oral diseases. The paper reviews the different oral ecosystems involving a variety of microbes and the balance between the growth of those microbes and the host health. In addition, the paper discusses the development of periodontal disease and dental caries according to plaque hypotheses. Relatively specific microfloras are associated with various types of periodontal conditions including *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis* and *Tannerella forsythia.* The genera *Streptococcu*s and *Actinomyces* are main indicators of periodontal health. The development of caries lesions appears to involve different bacterial succession. Mutans streptococci are implicated more with caries initiation, while lactobacilli appear to be related to progression of enamel and dentine lesions.

Key words: Plaque hypotheses, periodontal disease, dental caries, bacterial interactions.

INTRODUCTION

Oral ecosystems

An ecosystem consists of the microbial community living in a defined habitat and a biotic (that is, characterized by the absence of life) surrounding composed of physical and chemical elements. The oral ecosystem therefore is

*E-mail: idarout1959@gmail.com.

composed of the oral microorganisms and their surroundings, which constitute the oral cavity. The development of a community within an ecosystem usually involves a succession of populations. The process begins with the colonization of the habitat by pioneer microbial populations. In the oral cavity of newborns, streptococci including *Streptococcus mitis*, *Streptococcus oralis* and *Streptococcus salivarius* are the pioneer organisms (Kononen et al., 1992; Marsh and Martin, 1999).

Pioneer microorganisms fill the niche that is defined as the specific combination of physical, chemical and biological parameters that are necessary for the survival of a particular microorganism (Schonfeld, 1992). Recently, Marsh and Martin (1999) defined the niche as the function of an organism in a particular habitat. As a result of colonization, new populations may develop. The diversity of the pioneer oral community of the newborns increases during the first few months of life, several gram-negtaive anaerobic bacteria including *Prevotella melaninogenica, Fusobacterium nucleatum, Veillonella* and *Prevotella* spp. appear in the edentulous infants (Kononen et al., 1994a; Marsh and Martin, 1999). Succession ends when no additional niche is available for new populations. At this stage, a relatively stable assemblage of bacterial populations is achieved and called a stable or climax community. However, the concept of such community does not imply static conditions. The stability is based upon homeostasis, which indicates compensating mechanisms that act to maintain a steady-state condition by a variety of controls aimed at counteracting perturbations that would upset the steady state. The concepts of homeostasis and bacterial succession are important in oral microbiology. Some factors, such as a high-sucrose diet and poor oral hygiene, may cause an irreversible breakdown in the homeostasis of the oral ecosystem .

Oral habitats

The oral cavity contains several habitats, each being characterized by different physicochemical factors and thus supporting the growth of a different microbial community. The tooth can be described as a non-shedding hard surface that offers many different sites for colonization by bacteria below and above the gingival margin. In contrast, the oral mucosa is characterized by a continuous desquamation of its surface epithelial cells allowing rapid elimination of adhering bacteria. The mucosa that covers the cheek, tongue, gingiva, palate and floor of the mouth varies according to the anatomical site. The tongue with its papillary surface provides sites of colonization that are protected from mechanical removal. The area between the junctional epithelium of the gingiva and teeth referred to as the gingival crevice or periodontal pocket when its depth is > 3 mm, the latter also provides a unique colonization site that includes both hard and soft tissues.

Two important physiological fluids, the saliva and the gingival crevicular fluid constantly bathe the oral surfaces. These fluids are essential for the maintenance of the oral ecosystems by providing water, nutrients, adherence and antimicrobial factors. The supragingival environment is bathed by saliva, while mainly the gingival crevicular fluid washes the subgingival environment. The distribution of oral microorganisms differs qualitatively and quantitatively according to the habitat. For instance, mutans streptococci (predominantly *Streptococcus mutans* and *Streptococcus sobrinus* and for a less degree *Streptococcus cricetus* and *Streptococcus rattus*), in addition to *Streptococcus sanguis* are found in larger numbers on teeth, while *S. salivarius* is isolated mainly from the tongue. *S. mutans* and *S. sanguis* appear in the oral cavity only after eruption of the teeth (Smith et al., 1993).

MICROBIOLOGY IN ORAL HEALTH

Tooth surfaces

The initial intraoral deposit formed on a polished hydroxyapatite surface is a condensate of salivary proteins termed the acquired pellicle. Since this coating forms within a few minutes, it has been hypothesized that bacterial colonization would be unlikely to begin on any surface without the intital formation of a pellicle (Listgarten, 1994). Later, several studies have supported this hypothesis. The intitial colonizers (that is, bacteria that attach to this pellicle during the first hours) of the tooth surfaces are *Streptococcus* spp., *Actinomycetes* spp. and *Veillonella* spp. An attachment of these bacteria to pellicle macromolecules called receptors is mediated through proteoglycans as well as proteins in fimbriae and pili (adhesions). Once the intial adherence has occurred, the dental biofilm or dental plaque undergoes a process of maturation, including a succession of dominant microorganisms and an increase of thickness or biomass. The increase of plaque biomass occurs primarily by growth of the microorganisms rather than through the adhesion of additional microorganisms (Liljemark et al., 1997). Auschill et al. (2001) using a vital fluorescence technique combined with optical analysis by confocal laser scanning microscope studied the structure of dental biofilms. The authors noted that the spatial arrangement of microorgansims in dental biofilms showed voids outlined by layers of vital bacteria, which themselves were packed in layers of dead material. Plaque development and maturation have been described as a process of orderly succession of bacterial populations

from predominately gram-positive, facultative cocci to that represented by a gram-negative, anaearobic microbiota (Listgarten, 1994; Kolenbrander, 2000; Kolenbrander et al., 2010). It has recently been estimated that between 400 and 1000 microbial species may at some time colonize oral biofilms (Haffajee et al., 1999). Also recently, the development, microbial composition and treatment of dental biofilms have been extensively reviewed (Socransky and Haffajee, 2002).

Supragingival plaque

The predominant microorganisms of supragingival dental plaque are gram-positive, facultatively anaerobic bacteria, particularly *Actinomyces* spp. streptococci and *Capnocytophaga* spp. The gram-negative bacteria including *Veillonella* spp., *Prevotella* spp., *Tannerella forsythia,* and *P. gingivalis* have also been isolated from supragingival dental plaque but in low counts and proportions (Ximenez-Fyvie et al., 2000a). Once supragingival bacterial deposits have established an ecological niche of subgingival deposits begin to form.

Subgingival plaque

The composition of the subgingival microflora has been studied intensively because these deposits are involved in destructive periodontal diseases. In a healthy subgingival crevice, the total number of cultivable bacteria is relatively small [10^2 to 10^3 colony forming units (CFU)] and mainly dominated by gram-positive organisms, including *Actinomyces* spp. and streptococci (Darveau et al., 1997). Other species including *P. gingivalis, Porphyromonas endodontalis, Prevotella melaninogenica, Prevotella intermedia, Prevotella loescheii* and *Prevotella denticola* have also been isolated from healthy gingival crevices but in low levels and proportions (Marsh and Martin, 1999, Ximenez-Fyvie et al., 2000b). Haffajee et al. (1999) indicated that the counts and prevalences of *Actinomyces viscosus, S. sanguis, S. oralis, Veillonella parvula* and *Actinomyces odontolyticus* were high in healthy subjects. These species are for the most part considered to be host compatible or beneficial species.

Mucosal surfaces

The gingiva, palate, cheeks and floor of the mouth are colonized with few microorganisms (Theilade, 1990) that form oral biofilms. Streptococci constitute the highest proportion of the microbiota in these sites with a predominance of *S. oralis* and *S. sanguis*. The genera *Neisseria*, *Haemophilus*, *Prevotella, Actinomyces, Lactobacilus* and *Veillonella* have also been isolated in these sites. A higher bacterial density and diversity colonize the tongue (Bowden and Hamilton, 1998). According to these authors, the predominant members of the microbiota of the tongue are *S. salivarius, S. mitis* and *Veillonella* spp. In addition, *Peptostreptococcus* spp., gram-positive rods mainly *Actinomyces* spp., *Bacteroides* spp., and other gram-negative rods including *P. gingivalis, P. endodontalis, P. melaninogenica, P. intermedia, P. loescheii,* and *P. denticola* and spirochetes are commonly recovered from the tongue. Therefore, it was suggested that the tongue is a reservoir for micro-organisms that are implicated in periodontal diseases (van der Velden et al., 1986).

Saliva

Whole saliva has no distinctive microbiota of its own (Beighton, 1991; Munson et al., 2004), but is a reservoir for microorganisms regularly derived from dental plaque biofilms adhering to gingival crevices, periodontal pockets, the dorsum of the tongue and various other oral surfaces (van der Velden et al., 1986). The levels of microorganisms in saliva may reach up to 10^8 (Marsh and Martin, 1999) or 10^9 (Bowen, 1996) cells/ml saliva. Recently, Darout et al. (2002) have showed that the levels of *P. gingivalis, F. nucleatum, S. sputigena, S. sanguis* and *S. mitis* correlate significantly in whole saliva and subgingival plaque of adult subjects and that higher accuracy of detection and assessment of the levels of these bacteria in the oral cavity may be achieved by concurrent sampling of saliva and subgingival plaque.

MICROBIOLOGY IN ORAL DISEASES

Plaque hypotheses and periodontal diseases

Genco et al. (1988) demonstrated that organisms of the normal flora play a key role in gingivitis while exogenous organisms (*Aggregatibacter actinomycetemcomitans* and *P. gingivalis*) seem to be implicated in periodontitis. This is according to the exogenous plaque hypothesis (Genco, 1987). According to the non-specific plaque hypothesis, periodontal diseases in humans are due to plaque accumulation around the gingiva but not favouring any specific microorganisms. With the development of the specific plaque hypothesis, it was recognized that specific bacteria in subgingival plaque are the cause of the inflammatory periodontal diseases rather than the total number of bacteria (Loesche, 1999).

According to the Consensus Report (1996) World Workshop in Periodontics, there are sufficient data

incriminating *P. gingivalis, T. forsythia* and *A. actinomycetemcomitans* as etiologic agents for the various forms of periodontal diseases (Concensus Report, 1996). *P. gingivalis* is more frequently detected in severe adult periodontitis, in destructive forms of disease and in active lesions than in healthly or edentulous subjects. Its number is reduced in successfully treated sites but not in refractory sites and sites with recurrence of disease after therapy. *P. gingivalis* induces elevated systemic and local antibody responses in subjects with various forms of periodontitis (Mahanonda et al., 1991). The latter bacteria has been found in higher numbers in sites exhibiting destructive periodontal disease than in gingivitis or healthy sites (Lamster et al., 1994). *T. forsythia* has also been detected more frequently in active periodontal lesions (Dzink et al., 1988). The ecological plaque hypothesis (Marsh, 1991, 1994) may also be applied to explain the role of microorganisms in periodontal diseases. In the healthy gingival crevice, suspected periodontal pathogens such as *P. intermedia*, *A. actinomycetemcomitans*, *P. gingivalis* and spirochetes are undetectable or found in very small numbers. In the absence of oral hygiene, the accumulation of plaque can lead to inflammation and an increase in the flow of gingival crevicular fluid. This fluid may provide nutrients for bacteria and favour the growth of fastidious obligatory anaerobic gram-negative bacteria implicated in periodontal destruction. Cultures of subgingival plaque in serum allow the enrichment of suspected periodontal pathogens that were previously undetected in the primary inoculums (Ter Steeg et al., 1988). This finding may explain the observed succession of microorganisms from healthy gingiva and gingivitis to periodontitis and the difficulty in identifying specific etiologic agents in periodontal diseases .

Plaque hypotheses and dental caries

At neutral pH, mutans streptococci and lactobacilli are weakly competitive and constitute only a small percentage of the total plaque microbial community. Frequent consumption of fermentable carbohydrates may lead to frequent conditions of low pH in the plaque. Such conditions lead to decreased proportions of acid-sensitive bacteria like *S. sanguis*, *S. oralis*, and *S. mitis* and to increased proportions of mutans streptococci and lactobacilli. This bacterial shift is in accordance with the ecological plaque hypothesis mentioned earlier. Thus, a population shift predisposes a surface to dental caries. The increased numbers of cariogenic bacteria lead to the production of acid and proteolytic enzymes at a higher rate, enhancing the demineralization as well as degradation of the organic matrix of the tooth structure. The sequence of events explains the lack of total specificity in the microbial etiology of caries and the bacterial succession observed in longitudinal studies. The apparent absence of caries observed in the presence of high levels of *S. mutans* may be due to differences in flow rate, buffer capacity, or composition of saliva, fluoride content of dental hard tissues or to the presence of a high level of lactate-metabolizing and base-generating bacterial species in dental plaque (Marsh and Martin, 1992). Some studies suggest that the presence of *Veillonella parvula*, a lactate metabolizing bacteria, is associated with a lower prevalence of caries (Nyvad, 1993).

DISCUSSION

The microbial composition of the oral cavity is diverse and it varies from oral health and disease. In periodontal disease, the initiation and progression of periodontal tissue destruction are a complex process involving bacterial accumulation, invasion of gingival or periodontal tissue, release of bacterial substances, and host inflammatory responses (Genco, 1992). Spirochetes invade periodontal tissues in acute nececrotizing ulcerative gingivitis (Listgarten, 1965). *P. gingivalis* and *A. actinomycetemcomitans* have also been detected in crevicular epithelial cells of localized juvenile periodontitis and in adult periodontitis patients (Dibart et al., 1998). Moreover, Rudney et al. (2001) using fluorescent *in situ* hybridization and laser scanning confocal microscopy demonstrated intracellular *A. actinomycetemcomitans* and *P. gingivalis* in buccal epithelial cells of humans. The bacteria accumulating in the subgingival sites may release substances that penetrate the gingiva and cause tissue destruction directly, by the action of enzymes and/or endotoxins, or indirectly by induction of inflammation. The host inflammatory response to bacterial antigens is both protective and destructive in periodontal diseases. Tissue damage is caused by the release of lysosomal enzymes from phagocytes and by the production of cytokines (interleukin IL-1 and IL-6, and tumour necrosis factor TNF) by lymphocytes and monocytic cells that stimulate connective tissue cells to release matrix metalloproteinases including collagenases that stimulate bone resorption and inhibit bone formation. Among the bacteria regularly isolated from periodontal pockets, those producing such virulence factors generally include *A. actinomycetemcomitans* and species of *Porphyromonas*, *Prevotella*, *Fusobacterium, Capnocytophaga* and *Wolinella* species.

When oral hygiene is restored in gingivitis, the gingival tissue quickly returns to a state of health demonstrating that dental plaque according to non plaque hypothesis is responsible for gingival inflammation and is not a result of the disease. The bacterial load in plaque associated with gingivitis is 10^4 to 10^6 CFU with facultative gram-positive

bacteria still dominating, and an increase in the proportion of obligatory anaerobic gram-negative bacteria. The predominant gram-positive bacteria are *Actinomyces viscosus*, *Actinomyces naeslundii*, *S. sanguis*, *S. mitis* and *P. micros*. The gram-negative rods include *F. nucleatum*, *P. intermedia*, *Veillonella*, *Wolinella*, *Capnocytophaga* and *Haemophilus* spp. (Slots and Rams, 1992). Although it is not quite clear whether gingivitis is essential for the development of periodontitis, some species that predominate in periodontitis have been found in small numbers in gingivitis (Darveau et al., 1997). Chronic periodontitis is the most common form of advanced periodontal disease. The microbiota of this disease is extremely diverse with an increase in the total bacterial load to 10^5 to 10^8 CFU with a large number of obligatory anaerobic gram-negative rods and spirochetes (Moore et al., 1993; Darveau et al., 1997). The microbiota differs in composition between pockets within a patient and between patients. Haffajee et al. (1998) noted that predominant species of chronic periodontitis included *P. gingivalis*, *P. intermedia*, *T. forsythia*, *A. actinomycetemcomitans*, *W. recta*, *E. corrodens*, *T. denticola*, and *P. micros*. In addition, Tanner et al. (1998) indicated that *Campylobacter rectus* and *Selenomonas noxia* are prominent members of the subgingival microbiota in initial periodontal lesions in subjects with minimal attachment loss .

Aggressive periodontitis is closely associated with high numbers of *A. actinomycetemcomitans* (Slots and Schonfeld, 1991). Recently, it has been hypothesized that the aggressive form of periodontitis is the result of poly-infections by consortia of bacteria, similar to the accepted cause of adult forms of disease (Darby and Curtis, 2001). It has been indicated that periodontitis sites harbour high proportions of *A. actinomycetemcomitans*, *T. forsythia*, *P. gingivalis*, *C. rectus*, *E. corrodens*, *F. nucleatum*, *P. intermedia*, *P. melaninogenica*, *P. micros*, *Streptococcus intermedius* and spirochetes (Zambon, 1996). The presence of these bacterial species in subgingival plaque has been associated with certain clinical features with increasing prevalence in deep periodontal pockets (Wolff et al., 1993). The levels of *P. gingivalis*, *P. intermedia* and *A. actinomycetemcomitans* increase as probing depth increases and *P. gingivalis* is more abundant in suppurating sites (Haffajee and Socransky, 1994). Although spirochetes such as *T. denticola* are notoriously difficult to culture in the laboratory, these strict anaerobes may comprise more than 30% of subgingival microbiota of periodontitis patients.

Recently, it has been demonstrated that *P. gingivalis, T. forsythia* and *A. actinomycetemcomitans* are etiologic agents for the various forms of periodontal diseases (Consensus Report, 1996). This is in support of specific plaque hypothesis. The aforementioned periodontitis-associated species are always part of a large and varied microbiota found in subgingival plaque. Some of these species are not detected in certain periodontitis sites and can even be completely absent in cultures from multiple sites in an untreated periodontitis patient. The ecological plaque hypothesis may also be applied to explain the role of microorganisms in periodontal diseases (Marsh, 1991, 1994).

In the dental caries, the complexity of the bacterial community in cariogenic plaque in humans has made it difficult to determine a single bacterial agent of caries. However, there is considerable evidence that mutans streptococci (*S. mutans* and *S. sobrinus*) and *Lactobacillus* spp. are involved in the initiation and progression of caries (Loesche, 1986). These bacteria are able to rapidly metabolize carbohydrates into acid, primarily lactic acid, and to tolerate a low-pH environment. Cross-sectional studies demonstrated that a large number and isolation frequency of mutans streptococci and *Lactobacillus* spp. are associated with increasing prevalence of enamel lesions. In addition, most of the longitudinal studies have revealed that an increased level of mutans streptococci precedes the appearance of enamel caries (Loesche, 1986).

The increase in number of *Lactobacillus* spp. is generally slow, and it reaches a high level only after the lesion can be detected clinically (Burt et al., 1985). These findings suggest a microbial succession in which mutans streptococci are implicated in caries initiation and *Lactobacillus* spp. are implicated in caries progression (Nyvad, 1993). However, coronal caries also appears to develop in the absence of mutans streptococci and lactobacilli. Species such as *Actinomyces* spp., *S. mitis*, *Veillonella* spp. and *Candida* spp. have been associated with enamel caries (Nyvad, 1993). Other studies also suggest that the microflora of root surface caries is complex (Bowden, 1990). In addition to mutans streptococci and lactobacilli, a broad range of microorganisms may be isolated from root lesions, with *Actinomyces* occasionally constituting the predominant species (Bowden, 1990; Nyvad, 1993). Furthermore, high levels of *S. mutans* have been found in dental plaque without evidence of caries (Nyvad, 1993).

Frequent consumption of fermentable carbohydrates may lead to frequent conditions of low pH in the plaque. Such conditions lead to bacterial shift is in accordance with the ecological plaque hypothesis mentioned. Thus, a population shift predisposes a tooth surface to dental caries. The apparent absence of caries observed in the presence of high levels of *S. mutans* may be due to differences in flow rate, buffer capacity, or composition of saliva, fluoride content of dental hard tissues or to the presence of a high level of lactate-metabolizing and base-generating bacterial species in dental plaque (Marsh and Martin, 1992). Some studies suggest that the presence of *Veillonella*, a lactate metabolizing bacteria, is associated

with a lower prevalence of caries (Nyvad, 1993).

Conclusion

In the oral cavity, indigenous bacteria residing in dental plaque are relatively stable community of high species diversity, which may vary from site to site throughout the mouth. Many host-specific and environmental factors, in addition to oral hygiene and dietary habits which the subjects can regulate, appear to have the potential of modulating the composition of the oral microflora. Relatively specific microfloras are associated with various types of periodontal conditions including *A. actinomycetemcomitans, P. gingivalis* and *T. forsythia*. The genera *Streptococcu*s and *Actinomyces* are main indicators of periodontal health. The development of caries lesions appears to involve different bacterial succession. Mutans streptococci are implicated more with caries initiation, while lactobacilli appear to be related to progression of enamel and dentine lesions .

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